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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XIX JANUARY, 1943 NUMBER 1

CHANGES IN THE ACCESSORY SEX ORGANS OF THE MALE RAT AFTER ADMINISTRATION OF ESTRADIOL IN COMBINATION WITH PROGESTERONE OR DESOXYCORTICOSTERONE ACETATE*

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Several investigators have noticed that treatment with folliculoid (or "estrogenic") estrane derivatives may cause squamous metaplasia and even cornification of the epithelium in the prostates, coagulating glands, ampullary glands and seminal vesicles of rodents. At the same time these substances induce marked proliferation of the connective tissue and smooth muscle tissue in the walls of these glands.[1-4] In the rat this metaplasia is much more difficult to produce than in the mouse and the epithelium of the seminal vesicles appears to be practically resistant to this effect except in the region of the duct.[5,6]

Experiments on the mouse led Burrows [7] and de Jongh, Querido and Stolte [8] to conclude that the metaplasia normally produced by estrone can be prevented by simultaneous progesterone administration. In this connection it is perhaps worth mentioning also that according to Lipschütz, Vargas and Nunez [9] certain tumor-inducing effects of folliculoids are likewise counteracted by progesterone and desoxycorticosterone.

In the present communication we describe experiments on rats indicating that, at least under certain conditions, a special type of epithelial metaplasia can be produced by the combined administration of estradiol with either progesterone or desoxycorticosterone acetate, while similar treatment with any one of these steroids alone causes no such change.

EXPERIMENTAL FINDINGS

In our first experimental group 60 castrate male albino rats weighing 40 to 65 gm. were divided into six groups of 10. One group served as controls and received only peanut oil injections; the others were treated with various hormones or hormone combinations. The daily dose was

* Received for publication, March 2, 1942.

dissolved in 0.1 cc. of peanut oil and administered in two injections. All animals were castrated 2 days prior to the initiation of treatment. After 10 days of treatment the animals were killed and their sex organs weighed and examined histologically following fixation in Heidenhain's "Susa" mixture.

The upper portion of Table I summarizes the organ weights observed in the six groups of this experimental series. It will be seen that the weight of the seminal vesicles was increased by estradiol, but not significantly modified by progesterone or desoxycorticosterone acetate. In the combined administration of estradiol with progesterone or desoxycorticosterone acetate, the latter two compounds did not significantly influence the weight increase caused by estradiol. The weight of the prostate was slightly increased by progesterone and estradiol, but not by desoxycorticosterone acetate, and with combined treatment

TABLE I

Effect of Various Steroids On the Weight of the Accessory Sex Organs in Castrate Rats

Treatment	Dosage per day	Body weight in gm.		Seminal vesicle in mg.	Prostate in mg.	Preputial glands in mg.
		Initial	Final			
Oil		*55** (45–65)	*102* (82–124)	*9.7* (7.5–12)	*29* (21–35)	*23* (15.5–28)
α-estradiol	400 γ	*53* (40–60)	*77* (70–94)	*36* (24–45.5)	*40* (30–54)	*26* (13–39)
Progesterone	4 mg.	*53* (45–60)	*90* (75–128)	*11.5* (9–16.5)	*42.5* (31–53)	*33* (20–56)
Desoxycorticosterone acetate	4 mg.	*54.5* (45–65)	*83* (75–97)	*10* (6–13.5)	*28.5* (23.5–34.5)	*23* (18–26)
α-estradiol and desoxycorticosterone acetate	400 γ 4 mg.	*53* (40–60)	*82* (67–89)	*38* (24–46.5)	*40* (20–48)	*29.5* (15–38)
α-estradiol and progesterone	400 γ 5 mg.	*55* (40–62)	*88* (81–94)	*34* (25–43)	*62* (49–84)	*35* (24–47)
α-estradiol	100 γ	*53* (45–60)	*74* (55–95)	*29* (25–33)	*38* (33–44)	*24* (19–31)
Progesterone	10 mg.	*48* (40–55)	*90* (85–100)	*12.5* (12–14)	*113* (95–130)	*36* (26–53)
Desoxycorticosterone acetate	10 mg.	*40* (39–42)	*72* (70–75)	*9* (7–10)	*24* (17–31)	*17* (10–20)
α-estradiol and desoxycorticosterone acetate	100 γ 10 mg.	*57* (50–70)	*75* (60–100)	*27* (24–35)	*38* (27–55)	*27* (19–36)
α-estradiol and progesterone	100 γ 10 mg.	*55* (45–65)	*81* (75–90)	*31* (29–38)	*116* (78–146)	*37* (23–52)

* In each instance the average weight is given in italics, and beneath it the range is indicated in parentheses.

with estradiol and progesterone or desoxycorticosterone acetate the actions of each of these compounds appeared merely to be summated without any indication of potentiation. The preputial glands were markedly stimulated by progesterone only and the effect was not noticeably influenced by simultaneous estradiol administration.

Although the effect of the above three steroids on the gross weight of the accessory sex organs was merely additive, histological examination of the seminal vesicles revealed a curious change in the quality of the response to estradiol in the group in which this compound was administered in combination with progesterone. The epithelium of the seminal vesicles, which was not influenced either by progesterone or by estradiol alone, exhibited signs of marked proliferation and stratification in the group treated with both these compounds. Combined treatment with estradiol and desoxycorticosterone acetate did not elicit a similar epithelial proliferation in this experimental series.

It is well known that desoxycorticosterone acetate and progesterone are chemically and pharmacologically closely related compounds. Chemically the former differs from the latter only in the presence of an acetoxy- group in position C_{21}, and pharmacologically both substances possess luteoid and corticoid activity. It was therefore decided to repeat these experiments under otherwise identical conditions but giving a smaller dose of estradiol in combination with a larger dose of progesterone and desoxycorticosterone acetate. It was hoped that under such conditions the similarity in the effect of the latter two compounds would become evident and the curious type of epithelial metaplasia more readily demonstrable. The average weights of the accessory sex organs, as well as other details of this experiment, are summarized in the last five horizontal sections of Table I. It will be seen that even at the very high dosage level of 10 mg. per day, neither desoxycorticosterone acetate nor progesterone caused any significant change in the weight of the seminal vesicles, but while desoxycorticosterone acetate was also without effect on the weight of the prostates and preputial glands, progesterone definitely stimulated the latter two organs. In this, as in the first experimental series, the effect of the three steroids on the gross weight of the accessory sex organs was merely additive.

Histologically the lining of the seminal vesicles in the groups treated with progesterone and desoxycorticosterone acetate consisted of a single layer of epithelial cells which were perhaps slightly larger than those of the controls treated with oil, but never showed any sign of stratification. Similarly, none of the animals treated with estradiol alone showed stratification of the epithelium in the seminal vesicles, although they did exhibit the usual fibromuscular proliferation. On the other hand,

in the two groups receiving estradiol in combination with progesterone or desoxycorticosterone acetate, every animal exhibited marked proliferation and stratification of the epithelium and, although this never led to infiltration through the basement membrane into the muscular wall, it did, in many instances, completely obliterate the lumen of these glands. Histologically *we are not dealing with a squamous metaplasia, but merely with a stratification of the epithelium,* the cells of which retain their rather irregular cuboidal or polygonal shape. In certain instances we noted a good deal of sloughing of surface cells, which was reminiscent of holocrine secretion. Cornification was never observed. (Figs. 1 to 6.)

Discussion

Our observations indicate that even compounds such as progesterone and desoxycorticosterone acetate, which have no obvious effect on the gross weight of the seminal vesicles in the castrate male rat, possess the latent potentiality of influencing these structures. This fact can be made obvious by administering these compounds in conjunction with estradiol. We were surprised to note, because of the previously mentioned experiments of Burrows [7] and de Jongh, Querido and Stolte,[8] who prevented estrone from causing squamous metaplasia and keratinization by giving it in combination with progesterone, that combined treatment with progesterone or desoxycorticosterone acetate causes this type of epithelial metaplasia. It must be emphasized, however, that, unlike these investigators, we experimented on the rat and that the histological type of the metaplasia produced by us differs significantly from that seen by the earlier workers. In these short-term experiments combined treatment with progesterone or desoxycorticosterone acetate and estradiol never caused tumor formation, but it will remain for further experiments to show what the ultimate fate of this abnormal epithelial growth will be if treatment is continued over a long period.

We omitted a detailed description of the histological appearance of the prostates and preputial glands since they showed no noteworthy change except that progesterone stimulated their epithelium, an effect which was not modified by estradiol. Metaplasia was never observed in these organs. In contradiction to Hooker and Collins,[10] and in confirmation of the work of Paschkis [11] and Clausen,[12] we can say that desoxycorticosterone acetate is not a testoid compound; that is, it does not stimulate the accessory sex organs of the castrate male when given by itself. Greene, Burrill and Thomson [13] and Clausen [12] claimed that progesterone is definitely testoid as judged by its effects on the accessory sex organs of castrate males, but our experiments reveal that it

possesses only a stimulating action upon the prostate and preputial glands and fails to increase the weight of the seminal vesicles.

Summary

Experiments on immature castrate albino rats indicate that treatment during 10 days with estradiol, progesterone, or desoxycorticosterone acetate alone causes no significant change in the epithelium of the seminal vesicles, but if the same dose of estradiol is given during the same time in combination with either progesterone or desoxycorticosterone acetate, marked proliferation and stratification of the epithelium of the seminal vesicles is readily and consistently induced.

As an incidental finding it is noted that in immature castrate rats desoxycorticosterone acetate in doses up to 10 mg. per day fails to increase the weight of the seminal vesicles, prostates, or preputial glands. Progesterone enlarges the prostates and preputial glands under similar conditions, but fails to increase the size of the seminal vesicles even at this high level of dosage.

The expenses of this investigation were defrayed through the Blanche E. Hutchinson Fund of McGill University, and the steroid compounds were kindly donated by Drs. E. Schwenk and G. Stragnell of the Schering Corporation of Bloomfield, New Jersey.

REFERENCES

1. David, Károly; Freud, John, and de Jongh, S. E. Conditions of hypertrophy of seminal vesicles in rats. II. The effect of derivatives of oestrone (menformon). *Biochem. J.*, 1934, **28**, 1360–1367.
2. Freud, John. Wirkung der Geschlechtshormone auf die Präputialdrüsen. *Acta brev. Neerland*, 1933, **3**, 123.
3. Freud, John. Conditions of hypertrophy of the seminal vesicles in rats. *Biochem. J.*, 1933, **27**, 1438–1450.
4. de Jongh, S. E. Paradoxe Wirkungen von Follikelhormon (Menformon) bei männlichen Tieren; ihre Beeinflussbarkeit durch männliches Hormon. *Arch. internat. de pharmacodyn. et de thérap.*, 1935, **50**, 348–378.
5. Gaarenstroom, J. H., and de Jongh, S. E. The effect of diethylstilboestrol in the male organism. *Acta brev. Neerland*, 1939, **9**, 178–181.
6. de Jongh, S. E., and van der Woerd, L. A. Der Einfluss des Lebensalters auf die Art und den Umfang der paradoxen Oestronwirkungen. *Acta brev. Neerland*, 1939, **9**, 21–25.
7. Burrows, Harold. A protective action of progesterone on the genital organs of male mice. *Nature*, 1936, **138**, 164.
8. de Jongh, S. E.; Querido, A., and Stolte, L. A. M. Paradoxical effects of oestrone in male animals. IV. The inhibition of the paradoxical effect by progesterone. *Arch. internat. de pharmacodyn. et de thérap.*, 1939, **62**, 390–398.
9. Lipschütz, Alexander; Vargas, Luis, Jr., and Nunez, Carlos. Comparative antitumoral action of desoxycorticosterone acetate and testosterone propionate. *Proc. Soc. Exper. Biol. & Med.*, 1941, **48**, 271–274.
10. Hooker, C. W., and Collins, V. J. Androgenic action of desoxycorticosterone. *Endocrinology*, 1940, **26**, 269–272.

11. Paschkis, K. E. Androgenic action of desoxycorticosterone acetate. *Proc. Soc. Exper. Biol. & Med.*, 1941, **46**, 336–338.
12. Clausen, H. J. The effect of progesterone and desoxycorticosterone on the accessory sex organs of the male guinea pig. *Anat. Rec.*, 1941, suppl. **79**, 14–15.
13. Greene, R. R.; Burrill, M. W., and Thomson, D. M. Further studies on the androgenicity of progesterone. *Endocrinology*, 1940, **27**, 469–472.

DESCRIPTION OF PLATE

PLATE 1

FIG. 1. Seminal vesicle of castrate control animal treated with oil, showing poor development of epithelium and of fibromuscular coat.

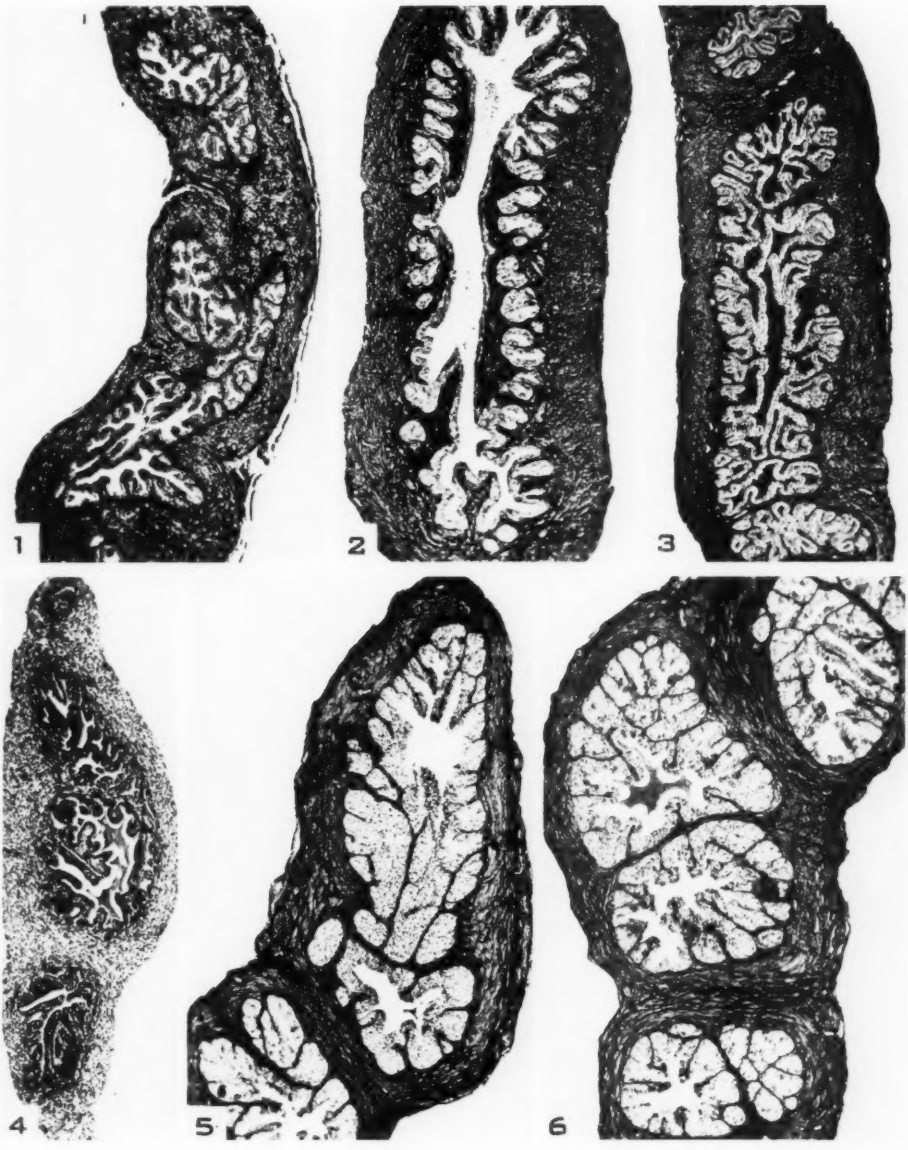
FIG. 2. Seminal vesicle of castrate rat treated with estradiol, showing proliferation of fibrous tissue and musculature without any significant change in the epithelium.

FIG. 3. Seminal vesicle of castrate rat treated with progesterone. The epithelium is slightly higher than in the control but forms a single layer only.

FIG. 4. Seminal vesicle of castrate rat treated with desoxycorticosterone acetate. The epithelium is slightly higher than in the control but forms a single layer only.

FIG. 5. Seminal vesicle of castrate rat treated with estradiol and desoxycorticosterone acetate, showing marked proliferation and stratification of the entire epithelial surface.

FIG. 6. Seminal vesicle of castrate rat treated with estradiol and progesterone, showing stratification of epithelium similar to that seen in Figure 5.



Masson and Selye Accessory Sex Organs Altered by Steroids

THE PRODUCTION OF CIRRHOSIS IN THE LIVER OF THE NORMAL DOG BY PROLONGED FEEDING OF A HIGH-FAT DIET *

By I. L. Chaikoff, M.D.,† K. B. Eichorn, M.D., C. L. Connor, M.D.,‡ and C. Entenman, Ph.D.

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The first experimental indication that the long-continued presence of large amounts of fat in the liver gives rise to fibrosis was obtained in the completely depancreatized dog kept alive with insulin.[1] When maintained on a diet of lean meat, sucrose, salts and vitamin supplements, depancreatized dogs develop fatty livers.[2] Although as much as 32 per cent of fatty acids may appear in the liver as early as 3.5 weeks after excision of the pancreas, the rate of infiltration of fat in the liver is highly variable.[3] It required about 16 weeks for all livers to attain a fatty-acid content in excess of 15 per cent. Once attained, however, fatty livers remain in such animals for long periods, and in one dog such a liver was observed as late as 3 years after pancreatectomy. But if the animals survived long enough, a spontaneous decline in the fat content of the liver might occur. In three dogs that survived from 4.2 to 5.5 years, nearly normal percentages of fatty acids were found, although the total amount of fatty acids present was still in excess of the normal because the size of the liver failed to regress as the fat left it.

The earliest evidence of hepatic fibrosis in a depancreatized dog was observed at an interval of 1.5 years after pancreatectomy.[1] Hepatic fibrosis developed in 8 of 16 dogs that had been kept alive from 2.6 to 5.5 years. In 4 of these, well advanced portal cirrhosis of the liver was found. By the time the most severe type of cirrhosis appeared, the fat content of the liver had been reduced to normal, and these livers showed little or no evidence that a marked infiltration had preceded the fibrosis.

The present report demonstrates that fibrosis and cirrhosis of the liver develop in *normal* dogs when their livers are kept excessively fatty by the continued administration of a high-fat diet.[4] This new observation involving fatty livers of dietary origin, together with that previously made on the depancreatized dog[1]—that the fatty liver occurred even though the diet fed was relatively low in fat—establishes a high

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‡ Deceased.

fat content in the liver as an important causative agent in the production of hepatic cirrhosis.

Experimental Findings

The dogs recorded in this study were fed twice daily, at 8:00 a.m. and at 4:00 p.m., a mixture of lean meat, lard and bone ash. Each dog received daily 7 gm. of lean meat and 10 gm. of lard per Kg. of its initial body weight, and 1 to 3 gm. of bone ash with each meal. Vitamins A and D were supplied as cod-liver oil, and the vitamin B complex as Galen B (Vitab, type II, liquid *). One gm. of Cowgill's salt mixture [5] was added to the evening meal.

Considerable variation was observed in the appetites of the dogs for this high-fat diet. All food not voluntarily eaten was force-fed. The dietary intake of each dog is recorded below:

Dog F-3. Ate well throughout experiment. Vomited only four times.

Dog F-6. Ate well for first 89 weeks, without vomiting. For the next month it was force-fed but vomited frequently. It then regained its appetite and continued to eat well, with only infrequent vomiting, until the 159th week. For the remainder of its period of survival it had to be force-fed every other meal, but vomited infrequently.

Dog F-20. Was force-fed throughout the period of observation. No vomiting occurred for the first 5 months, but for the next 2 months it vomited after almost every meal. No vomiting was then observed until the last 8 days of survival.

Dog F-28. Survived for 155 weeks. With the exception of 7 weeks it received the high-fat diet during the entire period of observation. The feeding of the high-fat diet was interrupted on two occasions, at 8 months and 15 months after the start of the experiment. During these two interruptions (7 weeks in all) it received a lean-meat diet supplemented with bone ash, Cowgill's salt mixture,[5] cod-liver oil and Galen B. Force-feeding of the high-fat diet was resorted to almost entirely. Vomiting occurred only infrequently.

Dog F-29. Force-fed throughout; no vomiting.

Dog F-33. Ate well for first 2 months. Force-feeding was necessary thereafter, except for occasional meals. Vomited infrequently.

Dog F-39. Ate well for first 2.5 months. Force-fed thereafter. Vomited infrequently.

Dog F-40. Ate well for first 15 days. Force-fed every meal thereafter.

Dog F-41. Ate well for first 2 months. Thereafter was force-fed the high-fat diet except for short intervals of 3 and 2 weeks, during which

* This concentrate was generously supplied by the Vitab Corporation, Emeryville, Calif.

time fat was excluded. No vomiting occurred until the last 18 days, when this became frequent.

Dog F-43. Force-fed throughout. No vomiting.

Dog F-44. Ate well for first 2 months. Force-fed thereafter. Vomited only during last 3 days. Fat removed from diet for 1 month, *i.e.*, between 4th and 5th month.

Dog F-45. Force-fed throughout. No vomiting.

Dog F-46. Force-fed throughout. Vomited infrequently during last month.

Dog F-48. Ate well for first 6 months. Force-fed thereafter. Vomited every third meal during last 10 days of its survival.

Dog F-49. Ate well during first 10 months. Force-fed every meal thereafter. Vomited occasionally during first 8 months; not at all thereafter.

Dog F-50. Ate well for first 8 months. Force-fed thereafter. Vomited frequently during entire period of observation.

Dog F-59. Ate well for first 2 months. Force-fed thereafter. No vomiting for first 5 months, but vomited frequently during remainder of period.

The following stains were used for microscopic examination of the livers: hematoxylin and eosin; aniline blue for connective tissue; phosphotungstic acid and hematoxylin for connective tissue; hematoxylin and van Gieson's for connective tissue; sharlach R for fat. Hematoxylin and van Gieson staining for connective tissue was found most satisfactory for black and white photographic reproduction.

The method employed for the determination of fatty acids has been described elsewhere.[6] A mixed sample of the whole liver was used for this analysis.

Results

The pathologic changes observed in the livers of the 17 dogs recorded in Table I have been grouped according to the progress of the lesion. It should be recognized, however, that very sharp distinctions cannot be made because of imperceptible transitions from one phase to another. In group I have been recorded the livers showing the earliest changes; this consisted of fatty infiltration of the liver cells. Group II includes the livers that showed an increased prominence of the intralobular or intercellular connective tissue, as well as those in which an actual fibroblastic proliferation and diffuse cirrhosis were observed. Livers showing nodular cirrhosis have been placed in group III; this resulted from a regeneration of liver cells that had been divorced from their normal portal and central relationship by the fibrosis. Five of the livers fall into group I, 10 into group II and 2 into group III.

TABLE I
Summary of Dogs

Dog no.	Sex	Duration of experiment	Died (D) or sacrificed (S)	Weight of dog			Liver			Group classification
				Initial (Kg.)	Maximum (Kg.)	Final (Kg.)	Weight (gm.)	Total fatty acids (per cent)	Gross and histologic appearance	
F-3	F	5 wks.	S	7.0	7.2	7.1	325	30.0	Marked fatty infiltration; no fibrosis; very little glycogen	I
F-6	F	3 yrs.+26 wks.	S	5.6	7.9	4.5	...	5.4	Very nodular liver; cirrhosis with hyperplasia	III
F-20	F	36 wks.	D	12.1	15.5	13.7	485	12.4	Pale fatty liver; good cirrhosis with bile retention	II
F-28	M	2 yrs.+51 wks.	S	7.6	9.0	8.0	251	20.9	Fatty liver; early slight fibrosis	II
F-29	F	15 wks.	D	8.2	8.2	7.6	943	35.4	Fatty green liver; early fibrosis and bile retention	II
F-33	F	37 wks.	D	6.6	7.5	7.5	475	25.4	Fatty, slightly green liver containing excess fibrous tissue	II
F-39	M	32 wks.	D	9.7	11.4	11.2	467	14.0	Fatty liver with well advanced cirrhosis throughout	II
F-40	M	10 wks.	D	6.1	6.5	6.1	410	25.7	Fatty liver with marked bile retention; no fibrosis	I
F-41	F	1 yr.+3 wks.	D	11.2	16.9	12.8	695	20.2	Liver green, fatty, firm and granular, showing advanced cirrhosis	II
F-43	F	24 wks.	D	6.6	8.6	7.1	448	27.4	Fatty liver without fibrosis	I
F-44	F	42 wks.	D	7.0	8.6	8.0	423	16.4	Fatty liver with diffuse fibrosis	II
F-45	F	13 wks.	S	9.1	12.2	12.1	443	37.6	Fatty liver; no fibrosis	I
F-46	M	13 wks.	S	14.1	16.8	16.8	426	23.6	Fatty liver without fibrosis	I
F-48	F	1 yr.+3 wks.	D	10.1	15.0	10.7	1600	17.0	Fatty green liver; advanced cirrhosis	II
F-49	M	1 yr.+7 wks.	S	11.4	15.1	10.5	720	13.6	Fatty green liver; cirrhosis with adenomatous regeneration	III
F-50	F	17 wks.	D	7.7	8.8	7.4	1020	46.6	Fatty liver with bile retention; early, diffuse fibrosis	II
F-59	M	23 wks.	S	10.0	12.9	10.0	1079	37.4	Fatty green liver; early, diffuse fibrosis	II

Early Fatty Changes in the Liver
(*See Fig. 1*)

The liver of dog F-46 is representative of the accumulation of abnormal amounts of fat within the liver cells at a stage in which no increase in connective tissue has yet occurred. This animal was sacrificed after it had been maintained for 3.2 months on the high-fat diet. Its liver was not unusual in gross appearance except for its fattiness. It contained 23.6 per cent fatty acids.

Microscopically, the liver showed a peculiar type of fatty infiltration similar to that noted in other animals of this series. Fat was present in all liver cells, generally in fine vacuoles, so that it more nearly resembled glycogen storage. Some cells, however, contained large fat globules. The cells distended with vacuoles tended to be larger than if they had been filled with glycogen. With a fat stain (sharlach R) the livers of this group showed fat in very large amounts in practically every hepatic cell. The cell membranes were generally more conspicuous than normal, each cell being separated by a rather wide, deeply staining, limiting membrane. There was no visible increase in connective tissue.

Variations. In an animal sacrificed after only 35 days (F-3), the liver contained 30 per cent of fatty acids. The fat had a midzonal distribution although many whole lobules were affected. In an animal sacrificed after 5 months and 18 days (F-43), a hyaline atrophy of the cells was observed around the portal spaces. These particular cells contained neither fat nor glycogen, although elsewhere in the lobule practically every cell was filled with fat.

Fibrous Changes in the Liver
(*See Figs. 2, 3 and 4*)

The pathologic changes in the livers of this group ranged from a slight but definite proliferation of young connective tissue (which extended out from portal areas) to an extensive diffuse fibrosis that appeared to follow no particular arrangement or pattern. The earliest changes occurred in relation to the portal spaces. In more advanced cases thin strands of connective tissue surrounded many of the lobules and also extended *into* the lobules to the vicinity of the central veins. This fibrosis proceeded without regard to portal or central relationship, so that the fully developed picture was that of an aimless proliferation of fibrous connective tissue involving the entire liver and ignoring its normal architecture. The following description is representative of the group.

Dog F-48. This animal died after it had been maintained on the

high-fat diet for 1 year and 3 weeks. The liver was greenish yellow in color and smooth on the surface. The cut surface was mottled with bile, and the tissue throughout seemed firmer than usual. *Microscopically*, the liver showed considerable fat and a great deal of bile, the bile having collected in the interstitial tissue and in many cells. The marked proliferation of connective tissue, which was found throughout the liver, bore no relation to the portal areas or to the lobules. The lobular arrangement had entirely disappeared. The connective tissue had grown in no definite or characteristic manner but was found everywhere infiltrated between cells, sometimes in large amounts, sometimes in thin strands. This actively growing connective tissue had incorporated many degenerating liver cells as it infiltrated the normal tissue.

Nodular Cirrhosis of the Liver
(*Figs. 5, 6 and 7*)

The two livers of group III (F-6 and F-49) differed from those of the preceding group in having present a distinct nodular or adenomatoid hyperplasia of islands of liver cells. The fibrosis was general, as in the livers of group II. But the diffuse and aimless distribution of the connective tissue had so changed that now slender strands or thick bands of fibrous tissue completely surrounded masses of liver cells. These masses of liver cells were lacking in their usual portal or central connections. The nodules were of all sizes and were scattered throughout the liver. The following description is that of the liver of dog F-6. This animal was sacrificed after it was kept on the high-fat diet for about 42 months.

The gross appearance of the liver was striking. It had a yellowish tawny color and was extremely irregular in shape. Its borders were sharp except at the lower edge of the right lobe, where, because of the nodules present, the border was rounded. The surface was nodular, the nodules ranging in size from a few millimeters to 2 cm. in diameter. These projected above the surface of the liver as much as 1 cm. Some were practically extruded and connected with the liver by a bit of capsular material. The four lobes consisted entirely of adenomatous masses of liver tissue. On first impression it suggested a carcinoma of the hepatoma type, but on section it looked like a remarkable adenomatoid hyperplasia in a cirrhotic liver.

Microscopic Examination. Most of the liver cells appeared relatively normal, except that they were not aligned in cords and had lost their relation with central veins and portal areas. They resembled postmortem liver cells in that they contained little or no glycogen, this presumably having been hydrolyzed. The cells were grouped into

rounded masses by either thick or thin strands of connective tissue that gave the liver the adenomatous picture described above. These areas varied in size from microscopic to very large nodules observed grossly. The cells within adenomatous areas contained considerable fat in large or small vacuoles and were similar to the cells in the smaller nonnodular patches of diffuse fibrosis. In some nodules there had been a diffuse proliferation of connective tissue; as noted above, these did not have the normal liver structure and seemed obviously to be regenerated hyperplastic liver tissue. Some cells contained two nuclei, but mitotic figures were not seen. Bile was present in large inspissated clumps, distended canaliculi and filled some of the smaller bile ducts. In the connective tissue radiating from portal areas there were many newly formed bile ducts, some with several layers of epithelium. Many such ductlike structures had no lumina. The condition was a remarkably good example of diffuse cirrhosis of the liver with bile-duct proliferation, bile retention and adenomatoid regeneration.

Discussion

The results of this investigation demonstrate the occurrence of a diffuse cirrhosis of the liver in dogs maintained on a high-fat diet. The first deviation from the normal was the accumulation of fat within the liver cells. This ranged from small, fine droplets to large globules which distended and distorted the liver cell. Practically every liver cell was affected. The cell boundaries became more distinct so that the cells were sharply outlined. This was accompanied by a hyaline atrophy or coagulative degeneration affecting cells adjacent to the portal veins. This picture was supplemented by the appearance of connective tissue fibers which first extended out from the portal spaces. Later there appeared a diffuse proliferation of connective tissue without regard to the normal architecture of the liver. In several cases the fibrosis was accompanied by bile-duct proliferation. In cases of longer duration, more or less distinct circumscribed nodules of regenerating liver cells were seen, while in two animals that survived more than 1 year a cirrhosis with discrete gross and microscopic nodules of hyperplastic liver tissue was observed.

It would seem that the diffuse fibrosis and cirrhosis observed in these dogs was produced by a low-grade injury maintained over a long period of time. That the injury to the liver was of mild degree was evident by the very slight or almost complete absence of inflammatory reaction. Neither inflammation nor infection appeared to be involved here, for no pathologic findings other than the liver fibrosis were observed in these dogs at autopsy. The coagulative degeneration of some of the liver

cells no doubt aided in stimulating fibrosis. Another factor to be considered here is interference in the hepatic circulation, since congestion was noted in several of the livers examined. Whether or not large amounts of neutral fat within the hepatic cell act as a *direct stimulus* to fibroblastic proliferation cannot be determined by the observations recorded here.

Summary

1. Diffuse cirrhosis of the liver was produced in the *normal* dog by the long-continued administration of a high-fat diet.

2. The hepatic lesions produced varied from early, slight or diffuse fibrosis to very nodular cirrhosis in which adenomatous regeneration had occurred.

3. The results of the present investigation point to continued fatty infiltration as an important causative factor in the production of liver cirrhosis.

References

1. Chaikoff, I. L.; Connor, C. L., and Biskind, G. R. Fatty infiltration and cirrhosis of the liver in depancreatized dogs maintained with insulin. *Am. J. Path.*, 1938, **14**, 101–110.
2. Kaplan, A., and Chaikoff, I. L. Liver lipids in completely depancreatized dogs maintained with insulin. *J. Biol. Chem.*, 1935, **108**, 201–216.
3. Chaikoff, I. L., and Kaplan, A. The distribution of fat in the livers of depancreatized dogs maintained with insulin. *J. Biol. Chem.*, 1937, **119**, 423–433.
4. Chaikoff, I. L., and Connor, C. L. Production of cirrhosis of the liver of the normal dog by high fat diets. *Proc. Soc. Exper. Biol. & Med.*, 1940, **43**, 638–641.
5. Cowgill, G. R. An improved procedure for metabolism experiments. *J. Biol. Chem.*, 1923, **56**, 725–737.
6. Chaikoff, I. L., and Kaplan, A. The blood lipids in completely depancreatized dogs maintained with insulin. *J. Biol. Chem.*, 1934, **106**, 267–279.

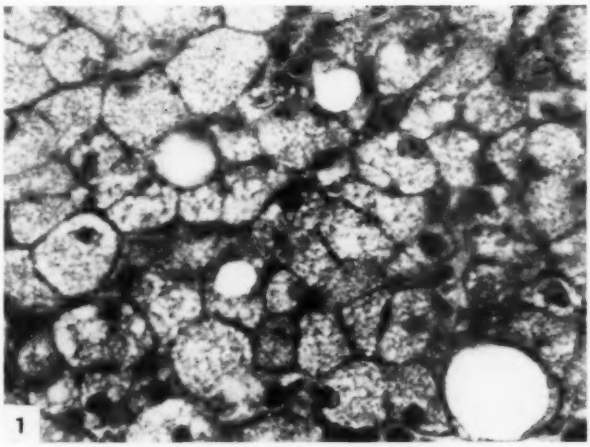
Description of Plates

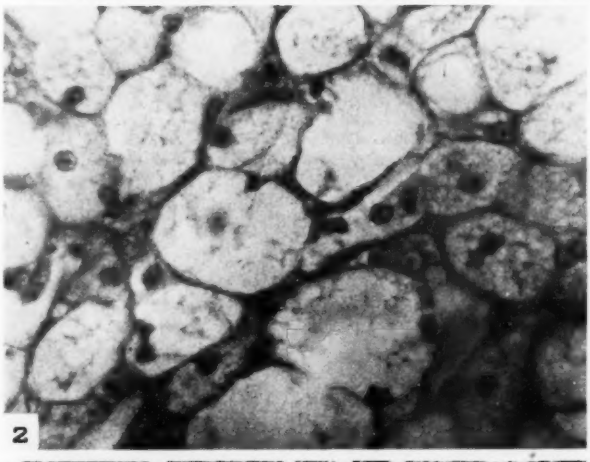
Plate 2

Fig. 1. Dog F-29. Peculiar type of fatty infiltration in large globules and in fine, small vacuoles resembling glycogen storage. This liver contained 35.4 per cent fatty acids. Hematoxylin and van Gieson stain. × 530.

Fig. 2. Dog F-59. Very fatty liver with early diffuse increase in connective tissue. This liver contained 37.4 per cent fatty acids. Hematoxylin and van Gieson stain. × 530.

Fig. 3. Dog F-44. Diffuse fibrosis disrupting the usual lobulated appearance of the liver. This liver contained 16.4 per cent fatty acids. Hematoxylin and van Gieson stain. × 130.





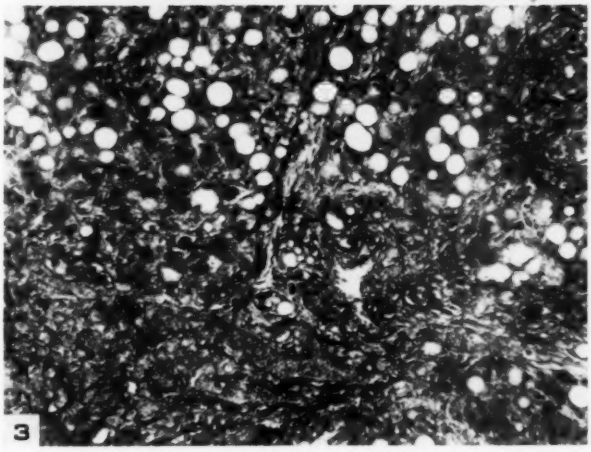


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PLATE 3

FIG. 4. Dog F-39. A good example of the pure fat type of cirrhosis. There is a great deal of relatively young and actively proliferating connective tissue. This liver contained 14 per cent fatty acids. Hematoxylin and van Gieson stain. × 150.

FIG. 5. Dog F-6. Cirrhosis of the liver with adenomatoid hyperplasia.

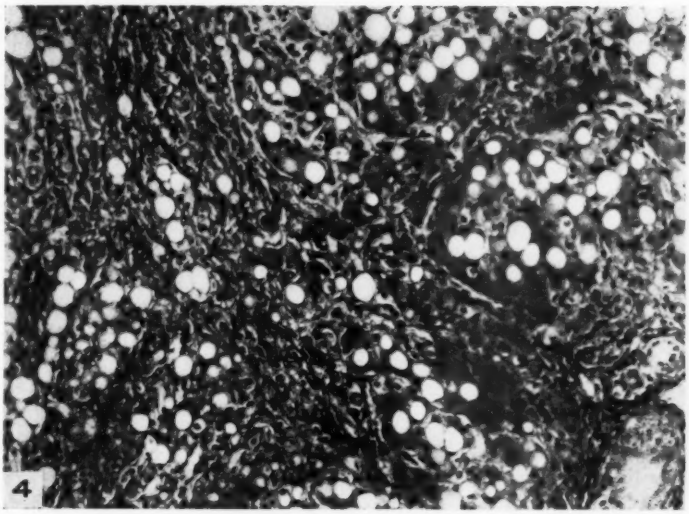

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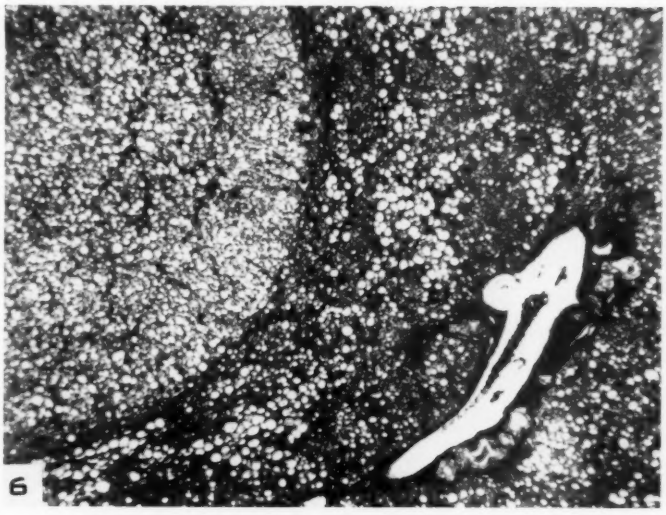
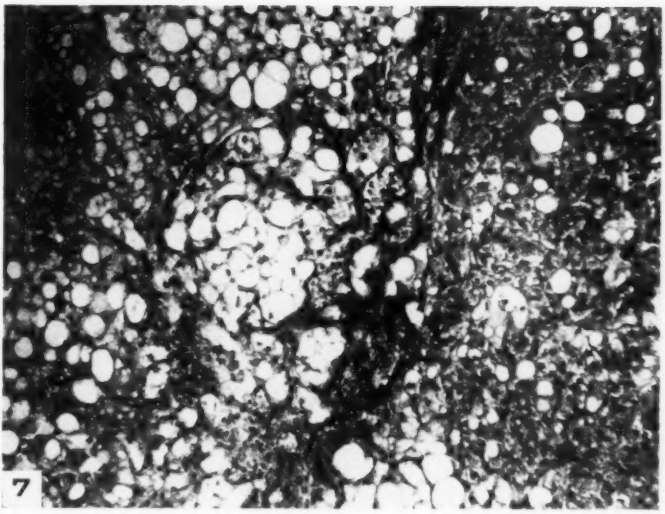
PLATE 4

FIG. 6. Dog F-49. Advanced cirrhosis of the liver with a circumscribed nodule isolated from the portal vein. This liver contained 13.6 per cent fatty acids. Hematoxylin and van Gieson stain. × 40.

FIG. 7. Connective tissue proliferation in the liver of dog F-49 showing an incipient nodule not fully encapsulated. Hematoxylin and van Gieson stain. × 150.





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PATHOLOGY OF STAPHYLOCOCCAL PNEUMONIA COMPLICATING CLINICAL INFLUENZA *

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From the latter part of December, 1940, through most of January, 1941, there occurred an outbreak of acute respiratory infections in and around Boston. Some of the epidemiological, clinical and laboratory aspects of this epidemic were reported by Pearson, Eppinger, Dingle and Enders.[1] These authors succeeded in isolating influenza A virus from typical uncomplicated cases. They also presented serological evidence that a large number of patients with pneumonia who were admitted to the Boston City Hospital during this time had had contact with influenza A virus, suggesting a possible relation between the two diseases. Among the patients with pneumonia who were seen during this period, there was an unusually large number in whom *Staphylococcus aureus* was the only, or the predominant, organism found in cultures from sputum, lungs, blood, or pleural fluid. A brief account of some of the latter cases has already been given.[2] We wish here to present the pathological features of the staphylococcal pneumonias observed during this influenza outbreak.

The association of the staphylococcus with certain cases of bronchopneumonia has been recognized since the influenza pandemic of 1889. During that epidemic and even earlier, necrosis of the bronchi and alveolar structure of the lung occurring in cases of influenzal pneumonia had been noted by a number of writers and staphylococci had been cultured from some cases.[3] Netter,[4] in a study of the bacteriology of bronchopneumonia, found staphylococci in 3 of 39 cases in adults from whom a single organism was obtained in pure culture and in 8 of 14 additional patients with mixed infections. Two of those with pure cultures of staphylococcus had multiple disseminated foci (abscesses?) and in the third there was "splenization" of the lungs. In infants and children he found staphylococci in 5 of 25 cases that yielded pure cultures and in 8 of 17 patients with multiple organisms. For the pneumonias complicating "la grippe" in 1889–90, he stated that there was unanimous agreement that the organisms were similar to those of other bronchopneumonias. He found staphylococci in 2 of 8 such cases, 1 associated with pneumococcus and the other with a streptococcus. Fraenkel [5] noted multiple abscesses in the influenzal bronchopneu-

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monias that occurred in 1889 and described them as characteristic of the pathology of staphylococcal infection in the lungs.

During the pandemic of 1918, the staphylococcus was not an important causative organism in any significant portion of the complicating pneumonias that were studied carefully by many different observers. There were some exceptions, notable among which were the cases reported by Chickering and Park [6] from Camp Jackson. In this camp the staphylococcus played the predominant rôle in the severe pneumonias observed during the influenza epidemic. Among 1409 patients with pulmonary complications, there were 385 deaths. Cultures, made from the lungs of 312 of the fatal cases, yielded a staphylococcus as the only or predominant organism in 153 instances. The disease in these cases was often fulminating, but if it was of long enough duration it was characterized by innumerable abscesses in the lung. In the patients who died earlier in the disease microscopical sections of the lungs showed intense congestion with rupture of alveolar walls and exudation of red cells and serum into the alveoli.

The report from no. 3 Canadian General Hospital [7] indicated that there the staphylococcus was next in importance only to the influenza bacillus. A study of 86 fatal cases of pneumonia in this hospital revealed all the various pathological findings described as characteristic of the pulmonary complications of influenza: bronchitis; peribronchial abscesses, scattered or clustered; bronchopneumonia, nodular or interstitial, with hemorrhage, congestion and edema; bronchiectasis, especially in the cases of longer duration; lobular pneumonia with abscesses; diffuse congestion and edema in the rapidly fatal cases; areas of collapse; hemorrhagic infarcts; emphysema with bullae; nonpurulent effusions in many instances and thin or thick pus in others; subpleural hemorrhages in most cases, and diffuse congestion of the trachea (but not the larynx). Bacteriological studies were done in 67 of these cases. *Staph.* (mostly *aureus*) was found as the only or predominant organism in 14 (21 per cent) of the lungs, as compared with 30 cases (45 per cent) in which influenza bacilli were obtained. Altogether, the staphylococcus was found in 51 cases (76 per cent) while influenza bacilli were identified in 90 per cent of the cases from one source or another. Cultures of the heart's blood were done in 50 of these cases and 25 were positive. *Staph. aureus* was obtained in 8 cases, the influenza bacillus in 2 and pneumococci or streptococci in the others. It is of interest that at the no. 5 General Hospital and no. 25 Stationary Hospital the staphylococcus was obtained from only 1 of 44 blood cultures and was not recovered from any of the cultures of the lungs or bronchi in 46 similar cases.[8]

Staph. aureus also played an important part in the complications

of influenza noted in the military hospital at Malta.[9] Among 50 cases, this organism was predominant in the sputum of 7 out of 9 fatal cases and in 9 patients who recovered from severe infections. In 9 of 11 autopsies there was evidence of infection with staphylococcus. All had extensive congestion and edema, but bronchopneumonia was not always widespread. In the involved areas the exudate was scanty, but there was a tendency to abscess formation. Empyema was found in 3 cases, all of which yielded *Staph. aureus*.

In a study by Winternitz, Wason and McNamara [10] of 82 autopsies in cases of influenza at New Haven, the staphylococcus was found alone in the lungs in only 1 case. It was recovered with other organisms in 19 cases, including 4 of the 34 acute cases, 9 of the 36 with necrotizing, and 6 of the 12 with organizing, pneumonia. Winternitz and his co-workers, in commenting on Chickering and Park's [6] observations, noted that necrotization and abscess formation were striking features of the pathology of the epidemic even when the staphylococcus was not found. They could demonstrate no relation between the bacteriological findings and the distribution or the type of the pneumonic process, and that was also the experience of the no. 3 Canadian General Hospital.[7]

The pathological picture in the lungs which was so consistently observed in the 1918 pandemic has also been thoroughly described by numerous other workers, notably MacCallum,[11] Goodpasture and Burnett,[12] Goodpasture [13] and Wolbach.[14] All of the latter writers, as well as Winternitz, Wason and McNamara,[10] described a hyaline membrane in the dilated bronchioles, in the alveolar ducts and sometimes in the subtended alveoli. There was always an associated necrotizing process of the tissue with an acute exudative reaction, hemorrhage, edema and necrosis of alveolar walls. Goodpasture [13] found the hyaline membrane in 70 per cent of cases examined at the height of the epidemic. In cases of secondary infection, however, the membrane was replaced by an extensive necrosis of the pulmonary tissue. Later, however, Brannan and Goodpasture [15] presented evidence to indicate that the hyaline membrane was not pathognomonic of influenza but probably represented a nonspecific toxic injury to capillaries. Farber and Sweet [16] demonstrated similar membranes in the lungs of infants who had aspirated amniotic sac contents. Farber and Wilson [17] also observed them in the lungs of children with lobar pneumonia or with streptococcal or tuberculous pneumonia, and they were able to reproduce the membrane experimentally in living or dead animals by the intratracheal inoculation of exudates followed by vigorous artificial respiration.

In the course of a mild epidemic of influenza that occurred in Eng-

land in December, 1936, and January, 1937, Stuart-Harris, Andrewes and Smith [18] found the commonest lesion to be "bronchiolitis." Because of the relative frequency with which influenza virus was isolated from cases with such lesions, they felt that this was essentially a true influenzal condition. The fulminating pneumonias, which were relatively few in that epidemic, they felt were almost certainly due to infection with both virus and *Staph. aureus,* a combination which was demonstrated in the lungs of 3 such fatal cases. The pathological findings in 1 of the latter cases were described by Scadding [19] and consisted essentially of fibrinous pleuritis, necrotizing tracheitis and bronchitis, and congestion of the lower lobes with less involvement of the apices. Histologically, there was a necrotizing alveolitis with hemorrhage filling the alveoli in infarctlike fashion. In limited areas there were small abscesses, 2 to 3 mm. in diameter; especially in these, but also throughout the affected areas, there were numerous small groups of gram-positive cocci.

Another single case in which both virus and *Staph. aureus* were isolated from the lung was reported by Stokes and Wolman.[20] This was a fulminating, edematous pneumonia without abscesses or hemorrhage. The patient showed a marked drop in neutrophilic leukocytes before death. The blood culture on the day before death yielded *Staph. aureus,* but culture of the heart's blood was sterile. This patient had been treated 3 weeks previously for a furuncle of the nose from which staphylococci were cultured, and there was a healing furuncle of the thigh at the time of the fatal illness.

Groups of sporadic cases of staphylococcal pneumonia not associated with definite outbreaks of influenza have been reported by a number of writers and appear to be more frequent in children than in adults.[21-28] In some of these cases the disease is preceded by symptoms suggesting clinical influenza, while in many the pulmonary lesions are associated with staphylococcal infection elsewhere in the body. Reimann [21] found *Staph. aureus* to be the predominant or only organism in 10 per cent of 700 cases collected from the literature. In Habbe's autopsies,[22] which are included in Reimann's series, staphylococci were found in pure culture in 7 of 93 cases of endemic bronchopneumonia and in 2 of 131 cases of lobar pneumonia, while among 20 cases of influenzal ("grippe") pneumonia they were found eight times in pure culture and five times as a mixed infection associated with streptococci, influenza bacilli, or pneumococci. Reimann [23] also described 6 cases of so-called primary staphylococcal pneumonia, 3 of which began after an influenzalike infection. All of the 6 cases presented clinical evidence of suppuration and abscess formation, but

only 2 of the patients died. The latter showed the typical pathological findings already mentioned.

Macgregor [24] reported autopsy findings in 10 cases of staphylococcal pneumonia in infants and children, 8 of whom were less than 1 year old. These cases occurred in one hospital during a period of 10 months. Four of the cases had early changes with massive consolidation in one or more areas of the lungs and serofibrinous pleural effusion. Hemorrhage and early onset of suppuration, especially of the bronchi and lymph vessels, were constant features. The other 6 cases were in a later stage and showed abscess formation, empyema and pyopneumothorax. Kanof, Kramer and Carnes [25] reported 37 cases in infants and children in which the staphylococcus was considered the etiological organism. In 25 of these patients the symptoms were similar to those of other pneumonias and the chief pathological findings were abscesses and empyema. The remaining cases had a picture predominately of sepsis with extrapulmonary foci of suppuration. There was no tendency for the cases to occur in epidemics. Cohen [26] reviewed some of the literature pertaining to staphylococcal infections of the respiratory tract. He estimated that in adults 1.6 to 1.8 per cent of all pneumonias and 10 per cent of bronchopneumonias are caused by staphylococci, while 10 per cent of all primary pneumonias in infants and children are due to this organism. Infants quickly develop empyema or pyopneumothorax, while older children, like adults, develop purulent bronchopneumonia but rarely have pleural complications. Gáspár,[27] reviewing the autopsy material of the Rochester General Hospital for 1933-1939, found the staphylococcus to be the only or the predominant organism in 38 of 144 pneumonias (26 per cent). Twenty of these 38 patients were in the first year of life and 8 others were between 2 and 10 years old. Bronchitis and tracheitis were constant. In the newborn the picture was one of hemorrhagic lobar or lobular pneumonia. In older babies there was often gray consolidation. At times there were pin-sized to pea-sized abscesses or consolidation arranged around bronchioles. Empyema was frequent and sometimes bilateral. Melton [28] reported 4 cases of staphylococcal lung infections in which recovery was attributed to treatment with sulfathiazole. Interestingly enough, these cases all occurred late in January and early in February, 1941, and in one of them the history suggested that the pneumonia was a complication of clinical influenza.*

The necropsy material to be presented consists of 8 cases; 4 of these

* Since this paper was completed, there have been reported two groups of cases similar to the acute cases to be described here. It is of note that the reported cases from Boston [29] as well as from Durham, North Carolina [30] occurred during the period of the influenza epidemic which we have studied.

are considered to be acute staphylococcal pneumonia on the basis of the clinical course and pathological findings, while the remaining 4 are classified, by contrast, as chronic, or organizing, staphylococcal pneumonias. One of the acute cases had a fulminating course; death occurred 6 days after the onset of symptoms of influenza and less than 36 hours from the time when the pneumonia presumably began.* In the other 3 acute cases death occurred from 6 to 8 days after the onset of influenza and 3 to 5 days after the appearance of severe symptoms of pneumonia. One of the latter patients was 65 years old; the other 3 were in their forties. In the 4 chronic cases, the duration of the severe respiratory symptoms was estimated at 15, 22, 25 and 46 days, respectively. The longest illness was in a patient of 50 years; the other 3 patients were over 70 years of age.

Brief Summaries of Case Histories

Case 1. Female, 46 years old. "Influenza" began January 4th, after the patient nursed her mother who had "grippe" from January 1st to 3rd. At 10:00 a.m., January 8th, some hoarseness and fever and rhonchi were heard over the sternum. At 8:00 p.m., sudden extreme dyspnea and cyanosis. At 10:00 p.m., dullness over right lower lobe, fine râles throughout both lungs; temperature, pulse and respirations increased. Scanty blood-streaked sputum, showing gram-positive cocci. Blood culture: *Staph. aureus*. Sulfathiazole and oxygen therapy begun. January 9th at 8:00 a.m., blood culture was negative; leukocytes, 800, and erythrocytes, 4,800,000; hemoglobin, 80 per cent. At 11:00 a.m., leukocyte count, 850. Remained semicomatose after 10:00 a.m.; lungs "filled up" rapidly; respirations became increasingly rapid and shallow and the patient died at 3:30 p.m.

Autopsy was performed 1½ hours postmortem. The pleural cavities showed no excess of fluid; there were a few old basal adhesions of the right lower lobe. The right lung weighed 1125 gm., the left, 400 gm. The lungs showed a fulminating hemorrhagic pneumonia. There was slight granulocytic hyperplasia of the bone marrow. There were no other significant pathological findings. Pure cultures of *Staph. aureus* were obtained from two sites in the right lung and one in the left lung. Culture of the heart's blood showed no growth.

Case 2. Female, 43 years old. Influenza began January 7th. On January 9th, the patient had shaking chills and cough with blood-tinged sputum. January 10th, there was marked dyspnea, prostration, increasing cough and presternal soreness. January 11th, at noon, crepitant râles were heard over the left lower lobe and sonorous râles bilaterally. The temperature was 103° F. Blood culture and sputum (mouse inoculation) yielded hemolytic streptococci. Leukocytes, 14,200. On January 12th, leukocytes, 11,000. There was a rapidly downhill course with increasing dyspnea, irregular, deep respirations, livid cyanosis and extension of the signs of consolidation to involve most of the lung. Death occurred at 1:00 a.m. on January 13th.

Autopsy was performed 9 hours postmortem. The pleural surfaces showed fibrinous and fibrous adhesions radiating from the hilus posteriorly, superiorly and inferiorly, and the left pleural cavity contained 500 cc. of thin amber fluid. The pericardial cavity was completely obliterated by fibrous adhesions, very dense and adherent except along the right auricle, where they could be separated easily and

* The autopsy in this case was performed by Dr. E. B. Fisk at the Waltham Hospital, and the clinical and pathological material was made available to us through the courtesy of Dr. George E. Currier and Dr. Sidney C. Dalrymple.

offered no obstruction to the vena cava. The right lung weighed 980 gm.; the left, 700 gm. Both lower lobes and the right middle lobe contained numerous discrete and confluent acute necrotizing abscesses arranged in relation to the bronchi. There was a necrotizing bronchitis and bronchiolitis. The other organs were not remarkable. Bacteriology: heart's blood, no growth; pleural fluid and left lower lobe, *Staph. aureus* and *Streptococcus hemolyticus;* right middle and lower lobes, pus from abscess in left lower lobe and the right pleura, all yielded only *Staph. aureus.* A ferret inoculated intranasally with a suspension from this patient's lung developed no signs of infection but proved refractory to subsequent inoculation with influenza A. The ferret's serum taken prior to the second inoculation protected mice against infection with this strain of virus.[1]

Case 3. Male, 46 years old. This patient had diabetes and had lost 40 lbs. during previous 8 months. January 9th, sudden onset of weakness. January 13th, slight pharyngeal injection, few crepitant and sibilant râles at the lung bases; temperature, normal; leukocytes, 6300. Diabetes readily controlled with insulin. January 15th, sudden marked prostration, dyspnea, fever and rapid pulse. Sputum culture: *Staph. aureus* predominating, and a few hemolytic streptococci. Sulfathiazole treatment begun. Dyspnea and cyanosis increased, signs in the lung extended rapidly and the patient died at 6:00 p.m., January 16th.

Autopsy was performed 17 hours postmortem. The pleural cavities contained a small amount of pleural fluid, 15 cc. in the left and 20 cc. in the right. There were fibrinous adhesions at both bases. The heart weighed 290 gm. and was normal except for slight coronary atherosclerosis. The right lung weighed 1160 gm. and the left, 960. Both lungs showed numerous acute abscesses, parenchymal hemorrhage and edema and necrotizing bronchiolitis. The liver weighed 1750 gm. and showed moderate fatty metamorphosis and minimal glycogen storage. The kidneys were pale and slightly increased in size (combined weight 420 gm.). Microscopically there was evidence of a moderate amount of glycogen storage in the tubular epithelium of the loops of Henle. Bacteriology: The heart's blood showed no growth; cultures from both lower lobes yielded a heavy growth of *Staph. aureus* and a few alpha hemolytic streptococci.

Case 4. Male, 65 years old. Influenza with cough and scanty sputum began January 6th. Cough increased January 9th and was accompanied by pain in the right side of the chest. January 12th, sudden marked dyspnea. January 13th, at 9:00 p.m., intense cyanosis, restlessness and air hunger. Areas of consolidation in both lungs, more on the right, with numerous scattered rhonchi. Temperature, 103.4° F.; pulse, 120; blood pressure, 170/80; blood culture yielded no growth. The patient died at 2:00 p.m. on January 14th.

Autopsy was performed 2 hours postmortem. The pleural surfaces of both lungs were covered with fibrinous exudate. There were 700 cc. of turbid yellow fluid in the right pleural cavity and 75 cc. of similar fluid in the pericardial cavity. The heart weighed 460 gm. and showed slight hypertrophy of the myocardium of both ventricles. Histologically, there was no evidence of pericarditis, nor did the heart muscle appear remarkable. The right lung weighed 1000 gm. and the left, 600. There was a healing infarct 3 by 4 cm. in diameter in the right upper lobe. The lungs showed hemorrhage, edema, necrotizing bronchitis and abscess formation. The liver weighed 2450 gm. and showed moderate central congestion. *Staph. aureus* was grown in pure culture from the lungs, and from the pleural and pericardial fluids.

Case 5. Female, aged 74 years. Admitted on January 12th, irrational. Severe dry cough since January 5th. There was Paget's disease of the skull, emphysema and auricular fibrillation. Blood pressure, 160/100. The patient was digitalized. There was an irregular low-grade fever. Leukocytes, 11,600. Sputum culture: *Staph. aureus,* type 17 pneumococcus and alpha streptococci. January 17th, notwithstanding signs of bilateral bronchopneumonia, the patient's condition was improving and

she was sitting up in bed. On January 19th, the patient again became irrational and thereafter the course was rapidly downhill with increasing dyspnea.

Autopsy was performed 45 hours postmortem. There were a few yellow-gray fibrinous bands on the pleura over the right upper lobe and some fibrinous patches over the lateral surfaces of both upper and lower lobes. The heart weighed 300 gm. and showed minimal atherosclerotic changes. The right lung weighed 770 gm., and the left, 500. Microscopically, there was evidence of pulmonary fibrosis with areas of walled-off abscess cavities connected with dilated bronchi. The process was most marked in both lower lobes posteriorly. The liver weighed 1400 gm. and showed slight engorgement of the central veins. The combined weight of the kidneys was 260 gm. and they showed a few scattered scars of healed pyelonephritis. The calvarium was thickened to 1.3 cm. and showed characteristic changes of Paget's disease; these changes were also seen in the vertebral column.

Case 6. Female, age 76 years. This patient was admitted January 24th with a history of a "cold," marked weakness and chest pain since January 18th. There were signs of consolidation of the right lung anteriorly, and extrasystoles. Blood pressure, 130/80. Sputum yielded *Staph. aureus* in pure culture; two blood cultures were negative. Leukocytes, 24,400. Treatment was with sulfadiazine for 6 days; digitalis was given in full doses. Temperature and leukocytes fell to normal by January 28th. February 3rd, leukocytes, 5500; irregular fever; dyspnea, and cyanosis. Râles in lungs increased progressively and patient died February 9th.

Autopsy was performed 18 hours postmortem. The right pleural cavity was obliterated by diffuse fibrous adhesions; scattered fibrinous adhesions were found in the left pleural cavity laterally. Heart weighed 260 gm. and showed minimal atherosclerotic changes. The right lung weighed 510 gm.; the left, 350 gm. Firm gray areas of fibrosis were found in the right upper and middle lobes, and moderate bronchiectasis in both lower lobes. Microscopically, there was moderate fibrosis of the lung parenchyma and moderately severe bronchiectasis. There were multiple benign polyps in the descending colon and rectosigmoid. There was microscopical evidence of moderate benign nephrosclerosis and a few scattered areas of healed pyelonephritis. Culture from the lungs showed *Staph. aureus* and influenza bacilli; the heart's blood showed no growth.

Case 7. Female, 50 years old. "Grippe," coryza, weakness and cough began December 24th. December 30th, sudden marked dyspnea and air hunger. Diffuse crepitant and sibilant râles and scattered areas of dullness and bronchial breathing at the bases. Irregular fever to 103° F., leukocytes 9000 to 18,000, constant and increasing dyspnea and cyanosis. Treatment with sulfathiazole, digitalis and oxygen was begun on admission and continued until the patient died on February 18th. She also received many small transfusions. Many sputum cultures showed *Staph. aureus* predominating in all specimens; type 14 pneumococcus was also obtained in the first specimen and hemolytic streptococci in the last two. Numerous blood cultures were negative except one taken on February 7th, which showed *Staph. aureus*.

Autopsy was performed 14 hours postmortem. There were bilateral fibrous pleural adhesions. The heart weighed 440 gm. The right auricle and ventricle were dilated and the latter showed a slight increase in thickness of the myocardium (0.5 cm.). Microscopically, miliary abscesses were seen in the myocardium. The left lung weighed 930 gm. and the right, 940. Both showed extensive fibrosis, bronchiectasis and thick-walled abscesses in the parenchyma, involving most of the lungs but especially marked posteriorly and inferiorly. The spleen weighed 500 gm. and contained a few small scattered abscesses. The liver weighed 2400 gm. and contained small abscesses in the portal and midzonal areas of the lobules. The combined weight of the kidneys was 340 gm. Microscopically, occasional glomeruli and neighboring collecting tubules contained acute inflammatory exudate and

there was an occasional small interstitial abscess in the pyramids. The other organs were negative. Cultures from many areas in the lungs showed *Staph. aureus* and hemolytic streptococci in varying proportions, and the heart's blood showed no growth.

Case 8. Male, 81 years old. Ill 2 to 3 weeks with prostration and vomiting. Admitted January 26th. Signs of diffuse bronchopneumonia, mostly in right lower lobe; temperature, 104° F.; pulse, 140, and leukocytes, 10,000. Sputum yielded a heavy growth of *Staph. aureus* and a few alpha hemolytic streptococci. Blood cultures were negative. There was improvement with drop in temperature and pulse rate after 2 days of therapy with sulfadiazine, digitalis and oxygen. February 1st, sudden rise in temperature, pulse and respiration; leukocytes, 27,000. The patient died February 3rd.

Autopsy was performed 11 hours postmortem. There were diffuse fibrous adhesions of both pleural cavities. The heart weighed 240 gm. and showed minimal atherosclerotic changes. The right lung weighed 500 gm. and the left, 560. The posterior lobes of both lungs and the right middle lobe showed considerable fibrosis of the parenchyma and bronchiectatic cavities. Acute inflammatory exudate was present in focal areas, especially about cellulose material in terminal bronchi. There was early organization of the exudate with foreign body giant cells in scattered areas, especially about the cellulose material. There was an old area of fibrosis at the right apex but no evidence of active tuberculosis. The kidneys showed the changes of benign nephrosclerosis. Cultures from both lower lobes yielded *Staph. aureus* and alpha hemolytic streptococci.

Acute Staphylococcal Pneumonias

The findings in the lungs of 3 of the acute cases were essentially similar. In them the involvement was bilateral and included portions of all the lobes, but it was most marked in the posterior and dependent portions. The lungs were increased in size and weight; the right lung varied from 980 to 1160 gm., the left from 600 to 960 gm. There was no definite exudate over the pleural surfaces in any of the cases, but in 2 of them there was a moderate amount of thin fluid in one pleural cavity. In 1 there were 500 cc. of clear amber fluid and in the other there were 700 cc. of fluid described as turbid yellow. Both these fluids yielded *Staph. aureus* on culture. In all 3 cases there were minimal fibrous adhesions at the posterior apical region, and in 1 case there were thin intralobar fibrinous strands. There was no evidence of subpleural hemorrhage, nor were any emphysematous blebs observed.

The lungs completely filled the pleural cavities and maintained their shape when they were removed from the thorax. Externally they presented mottled dull gray to dark red-purple anterior surfaces, which gradually blended into plum-colored and mottled posterior surfaces. In 1 case (Fig. 1) there was an area of old infarction in the right upper lobe which measured 3 by 4 cm., appeared dark red and had a uniform rubbery consistence on palpation. Scattered throughout the posterior lobes and in the inferior portion of the anterior lobes were

numerous gray areas which felt nodular and varied from 0.5 to 3.0 cm. in diameter. On the cut surface these nodular areas presented a homogeneous dirty gray appearance and exuded a mucoid exudate. When this exudate was scraped away, multiple abscess cavities were revealed which varied from 2 to 5 mm. in diameter, many coalescing to form honeycombed cavities measuring 1 to 2 cm. in diameter (Fig. 2). These abscessed areas were arranged, in general, in relation to the bronchi and bronchioles and usually were in direct communication with them. There was no evidence of any well defined wall to these abscesses, the contiguous parenchyma appearing markedly necrotic and fading into apparently intact alveoli filled with exudate. The intervening parenchyma presented a varied appearance; in some areas it was extremely wet and subcrepitant and exuded frothy bloody fluid, while in others the gray exudate-filled alveoli could be distinguished. Most of the parenchyma was involved and only about one-fifth to two-fifths of the lung was estimated to be air-containing. The trachea and bronchi were filled with plugs of yellow-gray, tenacious exudate. The pulmonary arteries and veins appeared grossly normal.

Sections were taken from various parts of the lungs for microscopical examination. There was extensive destruction of the usual architecture by an acute necrotizing process which was most marked in and about bronchi and bronchioles and involved adjacent alveolar tissue. The bronchi and bronchioles were almost completely denuded of their mucosa and their walls were infiltrated with acute inflammatory cells, chiefly polymorphonuclear leukocytes and histiocytes, and an abundance of fibrin (Fig. 7). The lumina of the ducts were filled with an acute inflammatory exudate. In 2 of the 3 acute cases there was no evidence of a hyaline membrane such as was so prominent in the influenzal pneumonias of 1918. The inflammatory process extended into the subtending alveoli with necrosis of the septa and resulted in the formation of abscesses, which varied in size from small miliary abscesses to large, conglomerate, necrotic masses (Fig. 6). There was no evidence of any limiting abscess wall of young connective tissue, nor was there any evidence of organization of the exudate. In some sections large veins were seen projecting into the abscesses. These veins were infiltrated with inflammatory cells and some of them contained fibrin thrombi in their lumina. Elsewhere, the blood vessels were markedly engorged, and in some areas they had actually ruptured and filled the alveoli with blood (Fig. 5). Other alveoli contained large amounts of edema fluid but no blood.

Large numbers of cocci in groups and clusters were seen in the exudate in scattered areas (Fig. 8). In case 2, smaller cocci in short

chains were also seen. The latter, however, were not present in any great numbers in the abscessed areas which showed the clumps of cocci. They were seen chiefly in alveoli which were filled with exudate but which had maintained their usual structure.

The pathological picture in the lungs in case 1 was somewhat different from that seen in the other 3 acute cases, and corresponded more closely to that of the most fulminating variety of influenzal pneumonia described in 1918. In this case the process involved chiefly the right lower and middle lobes and a small area in the left lower lobe. Grossly, the involved lobes were noncrepitant, heavy and moist, and exuded an abundance of bloody fluid. Microscopically, the picture in these lobes was predominantly one of hemorrhage and edema with very little fibrin and only a few polymorphonuclear leukocytes. Nevertheless, there were many groups of cocci seen. In spite of the meager leukocytic response, there was necrosis of alveolar walls. A few alveoli contained suggestive hyaline membranes. The bronchi showed marked edema and necrosis with very little cellular reaction. In uninvolved areas the alveoli were distended and some were disrupted, forming emphysematous blebs.

Cultures from the lungs yielded an abundant growth of *Staph. aureus* in every instance. In case 2, hemolytic streptococci were grown, in addition, from one of three lobes cultured, and a few alpha hemolytic streptococci were grown in case 4. Ferret inoculation of lung tissue from case 2 gave indirect evidence of the presence of influenza A virus.

The only other relevant finding in the acute cases was in the pericardial cavity in case 4. This contained 75 cc. of yellow turbid fluid, from which a pure culture of *Staph. aureus* was obtained. Microscopically, however, there was no evidence of acute pericarditis. Other incidental findings included an old obliterative fibrous pericarditis in case 2 and cardiac hypertrophy involving both ventricles in case 4. Cultures of the blood were made at the time of autopsy in every case and all remained sterile.

Chronic Staphylococcal Pneumonias

Four cases presented pathological findings which were similar, in most respects, to those of the "organizing" pneumonias described by Winternitz, Wason and McNamara [10] and others in relation to the influenza of 1918. The general appearance of the lungs was more or less alike in these cases. The weight of the lungs varied greatly: the right weighed from 510 to 940 gm. and the left from 350 to 930 gm. There were fibrous adhesions bilaterally, and these were moderately

extensive posteriorly, but there were no areas with exudate or fibrin. The pleural surfaces of the lungs presented a mottled appearance varying in color from a dark gray-red in the posterior portions to a gray-pink in the areas of emphysema which were most prominent in the upper lobes and in the periphery of the lungs. On palpation the basal portions of the lungs contained firm, rubbery nodular areas, especially posteriorly (Fig. 3). The upper lobes at their bases presented a similar, but less pronounced nodular change. In 1 case the major portion of the involvement was limited to the right upper and middle lobes; the right lower and entire left lung were only slightly involved.

On section the nodular areas were seen to be composed of large cavities with firm, well demarcated fibrous walls (Fig. 4). The majority contained yellow or gray-green mucoid exudate which could be expressed from the cavities. Most of the cavities communicated with bronchi which, in turn, were plugged with similar exudate. There was extensive fibrosis between the cavities, where the usual architecture was almost completely replaced by fibrous tissue. In areas which did not contain cavities the tissue either appeared emphysematous or was firm and of a dull red-gray color. There was no evidence of hemorrhage in the parenchyma. The cut surface was moderately dry. Trachea, bronchi and bronchioles contained gray-green, tenacious exudate which, when removed, revealed an apparently intact but erythematous mucosa. The pulmonary arteries showed no evidence of atheromatous intimal plaques, nor were they dilated. The pulmonary veins were not remarkable.

Microscopically there was evidence of extensive fibrous replacement of the alveoli. Large abscesses were in direct continuity with bronchi, forming bronchiectatic cavities. The bronchial walls were infiltrated by numerous plasma cells, scattered lymphocytes and histiocytes and rare foreign body giant cells (Fig. 9). The abscess cavities were filled with a more acute inflammatory exudate, and their walls showed some persistence of the necrotizing process. In 1 case some recently aspirated cellulose material was seen. In 3 of the cases there was an interstitial fibrosis in addition to chronic abscess cavities. The alveoli in the areas of interstitial fibrosis were lined by low cuboidal epithelium. There was evidence of regeneration of the bronchial mucosa (Fig. 10). A number of areas of regenerating mucosa appeared to be pinched off and imbedded in the contiguous parenchyma, forming small islets of epithelial cells. A similar regenerative process was described by Winternitz, Wason and McNamara [10] in the cases observed in the 1918 influenza epidemic.

The alveoli in some areas contained inflammatory exudate in different stages of organization. Large sheets of young connective tissue could be seen extending from one alveolus into another through septal defects. The alveolar capillaries were all dilated and filled with blood, as were the majority of the veins. The arteries in the areas where there was marked fibrosis and chronic inflammation showed a very early proliferative endarteritis, but no evidence of necrotizing arteritis.

In the cultures made at autopsy, *Staph. aureus* was the predominant organism recovered from the lung in all the cases. Alpha hemolytic streptococci were present in small numbers in 2 cases, beta hemolytic streptococci occurred in another and influenza bacilli were grown from 2 cases. The heart's blood showed no growth in any case.

The cardiac weights were within average range. There was a slight thickening of the right ventricular myocardium in case 7. The myocardium in this case contained small acute abscesses which were also seen in the spleen, liver and kidneys, suggesting blood-borne metastases.

The vertebral bone marrows in both the acute and chronic cases showed hyperplasia of both the red and the white blood cell series with normal maturation of the cells.

Comment

The pathological findings in these cases are similar, in most respects, to those described in the influenzal pneumonias of 1918. All stages are represented—the fulminating and the necrotizing varieties of the acute form and the organizing type in which death was delayed for longer than 2 weeks.

The cases presented are of interest mainly for two reasons: First, they represent the severest of the pneumonias that may be observed in the course of an epidemic of influenza which, from all other points of view, could be considered as comparatively mild. The first symptoms of respiratory infection in all these cases occurred within a period of 4 weeks, from December 24, 1940, (case 7) to January 18, 1941, (case 6), during which influenza was prevalent in and around Boston. In 5 of the cases there were typical symptoms of clinical influenza which began within a single week, between January 3rd (case 6) and January 9th (case 3), when the epidemic was at its peak.

Secondly, these cases are of special interest because they show clearly the important rôle played by *Staph. aureus* in the pulmonary complications of this epidemic. Other cases of pneumonia that occurred during the same period were of the usual varieties, both clinically and pathologically, and they were associated with the usual organisms

encountered in that season. Probably some of the latter patients also had clinical influenza, or at least contact with influenza virus, as indicated by immunological studies.[1] The staphylococcus, however, was unusually prevalent as an apparently important secondary invader in some cases of typical pneumococcal pneumonia that occurred at this time. It was the only or predominant organism in a large number of patients with clinical influenza, many of whom had pulmonary complications. Those among the latter who survived presented a variety of clinical pictures which have been described briefly elsewhere; [2] the pathological lesions in the fatal cases have been described here. The clinical features have been described in greater detail recently.[31]

In the experience of this hospital, staphylococcal pneumonias have been observed sporadically, especially in infants and young children and as a complication of staphylococcal infection elsewhere in the body. This is the first time that the staphylococcus has been identified in relation to a large number of apparently primary pulmonary infections within such a brief period.

Summary and Conclusions

The pathological findings in 8 cases of pneumonia in which *Staph. aureus* was the only or predominant organism have been presented. These cases all occurred during an epidemic of influenza and probably represent pulmonary complications of influenza. Three forms similar to those described among the influenzal pneumonias of 1918 were included; namely, (1) the acute fulminating, (2) the acute necrotizing and (3) the chronic organizing types. From this experience and from a number of reports in the literature, it is suggested that during an epidemic of influenza *Staph. aureus* may assume epidemic prevalence in some areas and give rise to severe pulmonary complications.

REFERENCES

1. Pearson, H. E.; Eppinger, E. C.; Dingle, J. H., and Enders, J. F. A study of influenza in Boston during the winter of 1940–1941. *New England J. Med.*, 1941, **225**, 763–770.
2. Finland, Maxwell; Strauss, Elias, and Peterson, O. L. Staphylococcal pneumonia occurring during an epidemic of clinical influenza. *Tr. A. Am. Physicians*, 1941, **56**, 139–144.
3. Leichtenstern, O. Influenza und Dengue. In: Nothnagel, Hermann. Spezielle Pathologie und Therapie. Alfred Hölder, Wien, 1896, **4**, pt. 1.
4. Netter. Étude bactériologique de la bronchopneumonie chez l'adulte et chez l'enfant. *Arch. de méd. expér. et d'anat. path.*, 1892, **4**, 28–65.
5. Fraenkel, Albert. Spezielle Pathologie und Therapie der Lungenkrankheiten. Urban & Schwartzenberg, Berlin, 1904, p. 533.

6. Chickering, H. T., and Park, J. H., Jr. *Staphylococcus aureus* pneumonia. *J. A. M. A.*, 1919, **72**, 617–626.
7. Tytler, W. H.; Janes, R. M., and Dobbin, G. M. Pathological and bacteriological findings in fatal cases of pneumonia during the influenza epidemic of October and November 1918. In: Studies of influenza in hospitals of the British armies in France, 1918. *Medical Research Council, Special Report Series No. 36,* His Majesty's Stationery Office, London, 1919, pp. 77–87.
8. Patterson, J. W.; Little, E. M., and Williams, S. E. Report on the bacteriology and pathology of 46 fatal cases of influenza. In: Studies of influenza in hospitals of the British armies in France, 1918. *Medical Research Council, Special Report Series No. 36,* His Majesty's Stationery Office, London, 1919, pp. 88–92.
9. Patrick, Adam. Note on *Staphylococcus aureus* septicaemia as a complication of influenza in an epidemic in Malta. *Lancet,* 1919, **1**, 137–138.
10. Winternitz, M. C.; Wason, I. M., and McNamara, F. P. The Pathology of Influenza. Yale University Press, New Haven, 1920.
11. MacCallum, W. G. The Pathology of Pneumonia in the United States Army Camps During the Winter of 1917–18. Monograph No. 10 of The Rockefeller Institute for Medical Research, New York, 1919.
12. Goodpasture, E. W., and Burnett, F. L. The pathology of pneumonia accompanying influenza. *U. S. Nav. M. Bull.*, 1919, **13**, 177–197.
13. Goodpasture, E. W. The significance of certain pulmonary lesions in relation to the etiology of influenza. *Am. J. M. Sc.*, 1919, **158**, 863–870.
14. Wolbach, S. B. Comments on the pathology and bacteriology of fatal influenza cases, as observed at Camp Devens, Mass. *Bull. Johns Hopkins Hosp.*, 1919, **30**, 104–109.
15. Brannan, Dorsey, and Goodpasture, E. W. The pathology of pneumonia caused by *Bacillus influenzae* during an inter-epidemic period. *Arch. Int. Med.*, 1924, **34**, 739–756.
16. Farber, Sidney, and Sweet, L. K. Amniotic sac contents in lungs of infants. *Am. J. Dis. Child.*, 1931, **42**, 1372–1383.
17. Farber, Sidney, and Wilson, J. L. The hyaline membrane in the lungs. I. A descriptive study. *Arch. Path.*, 1932, **14**, 437–449. The hyaline membrane in the lungs. II. An experimental study. *Arch. Path.*, 1932, **14**, 450–460.
18. Stuart-Harris, C. H.; Andrewes, C. H., and Smith, Wilson. A study of epidemic influenza: with special reference to the 1936–7 epidemic. *Medical Research Council, Special Report Series No. 228,* His Majesty's Stationery Office, London, 1938.
19. Scadding, J. G. Lung changes in influenza. *Quart. J. Med.*, 1937, **6**, 425–465.
20. Stokes, J., Jr., and Wolman, I. J. The probable synergism of human influenza virus and *Staphylococcus aureus* in a rapidly fatal respiratory infection. *Internat. Clin.*, 1940, **1**, 115–123.
21. Reimann, H. A. The Pneumonias. W. B. Saunders Co., Philadelphia & London, 1938, pp. 196–206.
22. Habbe, Karl. Zur Bakteriologie bei Lungenentzündungen des Menschen. *Deutsche med. Wchnschr.*, 1929, **55**, 1506–1508.
23. Reimann, H. A. Primary staphylococcic pneumonia. *J. A. M. A.*, 1933, **101**, 514–520.
24. Macgregor, A. R. Staphylococcal pneumonia. *Arch. Dis. Childhood,* 1936, **11**, 195–204.
25. Kanof, Abram; Kramer, Benjamin, and Carnes, Moses. Staphylococcus pneumonia. A clinical, pathologic and bacteriologic study. *J. Pediat.*, 1939, **14**, 712–724.

26. Cohen, Philip. Primary bronchogenic staphyloccoccic pneumonia. *M. Clin. North America,* 1938, **22,** 1473–1494.
27. Gáspár, I. A. A study of primary staphylococcic pneumonias occurring at the Rochester General Hospital. *New York State J. Med.,* 1941, **41,** 834–840.
28. Melton, G. Sulphathiazole in staphylococcal lung infections. *Lancet,* 1941, **2,** 522–523.
29. Michael, M., Jr. *Staphylococcus aureus* pneumonia with special reference to its occurrence as a complication of influenza. *J. A. M. A.,* 1942, **118,** 869–874.
30. Baker, R. D. Staphylococcal pneumonia during epidemic influenza in North Carolina. *South. M. J.,* 1942, **35,** 240–247.
31. Finland, Maxwell; Peterson, O. L., and Strauss, Elias. Staphylococcic pneumonia occurring during an epidemic of influenza. *Arch. Int. Med.,* 1942, **70,** 183–205.

DESCRIPTION OF PLATES

PLATE 5

FIG. 1. Left lung (case 2) showing extensive diffuse hemorrhage, edema and exudate. Confluent peribronchial abscesses are present in the lower lobe.

FIG. 2. Detail view of part of the lung shown in Figure 1. The major bronchi show acute confluent and necrotizing bronchitis.

FIG. 3. Left lung (case 7) showing marked distortion from extensive fibrosis and slight emphysema.

FIG. 4. Detail view of base of lower lobe of the lung shown in Figure 3. There is replacement of the usual structure by fibrous tissue and "honeycombed" cavities.

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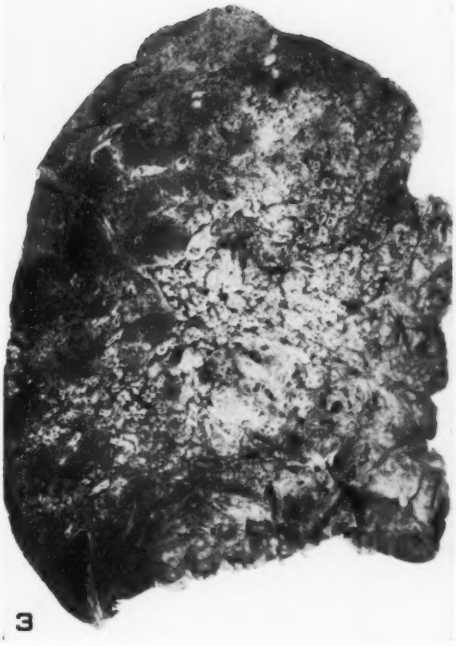

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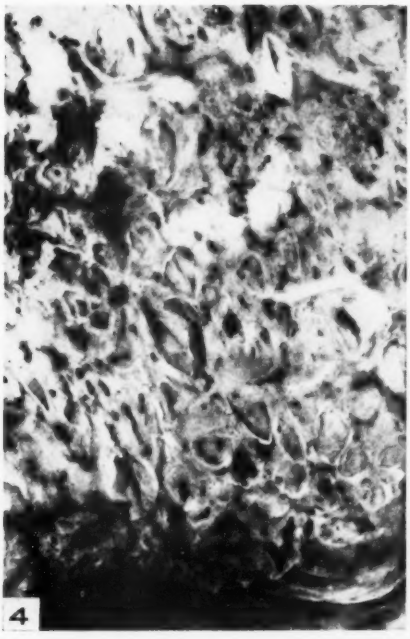


4

Wollenman and Finland

Pathology of Staphylococcal Pneumonia

Plate 6

Fig. 5. (case 2). Acute hemorrhage and edema fluid filling alveoli and destroying alveolar walls. Minimal exudate. Phloxine-methylene blue stain. × 70.

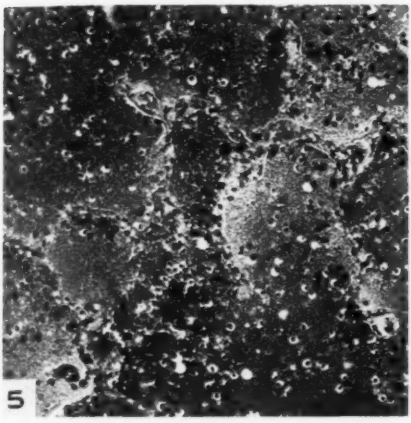
Fig. 6. (case 3). Acute abscesses of lung parenchyma. Here may be seen an hyaline membrane lining the alveoli in an area of trapped air. Phloxine-methylene blue stain. × 50.

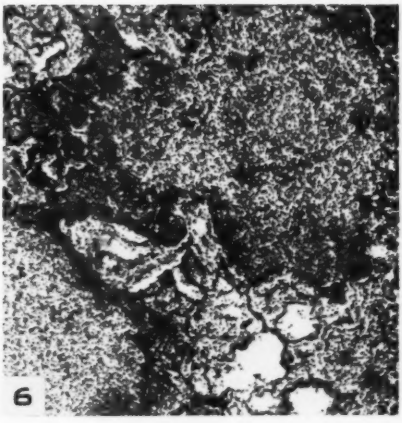
Fig. 7. (case 2). Acute necrotizing bronchitis. Phloxine-methylene blue stain. × 100.

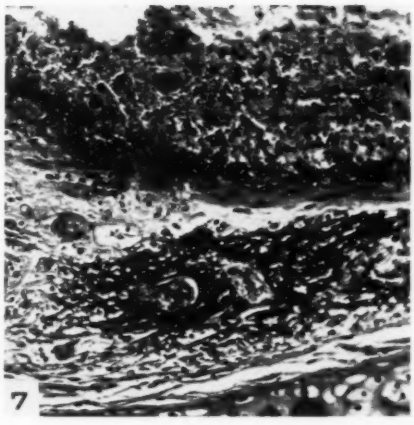
Fig. 8. (case 2). Large clumps of cocci in acute exudate. Gram-Weigert stain. × 750.

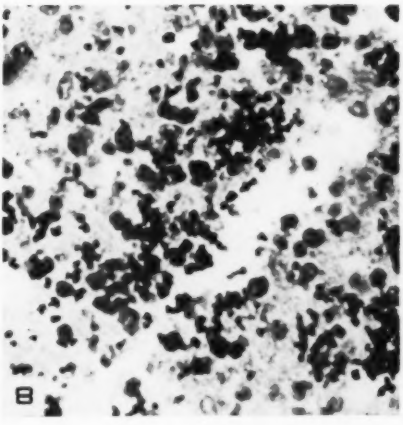
Fig. 9. (case 7). Extensive fibrous tissue replacement of lung tissue resulting in multiple small cavities. Phloxine-methylene blue stain. × 100.

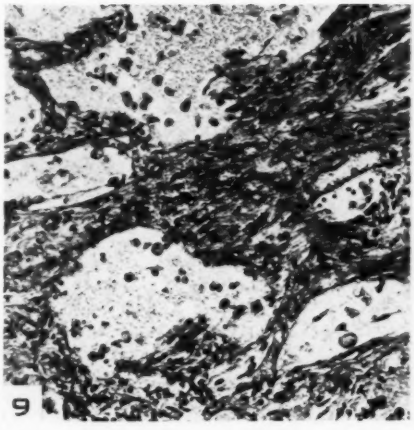
Fig. 10. (case 6). Proliferation of bronchial epithelial mucosa in areas of extensive fibrosis. Phloxine-methylene blue stain. × 100.











Wollenman and Finland Pathology of Staphylococcal Pneumonia

CHRONIC GASTRITIS

Its Relation to Gastric and Duodenal Ulcer and to Gastric Carcinoma*

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The term, gastritis, is commonly used in a restricted sense to designate a series of changes manifested chiefly in the mucosa of the stomach. Histologically, the process is characterized for the most part by excessive accumulations of lymphocytes and plasma cells and by atrophy and abnormal regeneration of the glands. These and other features have been described in detail by numerous writers as they occur in relation to gastric and duodenal ulcer and carcinoma of the stomach as well as in relation to gastritis alone (Konjetzny,[1] Faber [2]). The significance of the findings has been minimized, however, by reports of a high incidence of the same anatomic changes in individuals free of ulcer or carcinoma (Hamperl,[3] Hillenbrand [4]). The uncertainty and largely speculative consideration of etiologic factors emphasize that doubted significance.

A relatively large number of resected stomachs which presented the features of chronic gastritis in addition to the major lesion—ulcer or carcinoma—for which resection was performed stimulated interest in the problem. This material offered an opportunity to verify or disprove the character and distribution of the reported mucosal changes in relation to ulcer and carcinoma. In view of the reported high incidence of the same changes in otherwise normal stomachs, it was considered necessary to study a series of stomachs from patients free of manifest gastric disease. In this paper the findings in the surgical material will be reported and compared with the findings in control autopsy material.

Material and Methods of Investigation

A. Autopsy Material

The stomachs of 260 individuals free of manifest gastric disease served as controls. This material was obtained from autopsies performed by members of the Department of Pathology at the University of Minnesota, and the great majority were from the autopsy service of the University of Minnesota Hospitals. These were patients whose histories revealed no gastric symptoms except as related to the pre-

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senting illness and were otherwise unselected, except in so far as the autopsy experience may deal with selected cases. The reliability of the recorded historical data may be questioned in some instances, but it may be said that at least there were no serious gastric disorders among the group.

Microscopic sections were prepared from the lower antrum near or including, in most instances, the pylorus and from the midbody on or near the greater curvature in all cases. In addition sections were frequently taken from the upper antrum on the lesser curvature and from other portions of the body. Only specimens free from obscuring postmortem changes were used. Whether sampling of this kind adequately depicted the condition of the mucosa as a whole was tested by study of 30 specimens in greater detail. In these, long strips of mucosa were prepared according to the method used routinely for the surgical material (see below). These were compared with the sample sections. They showed the same features and no case had to be reclassified. While undoubtedly some focal changes may be missed, the sampling seems to provide a reasonably accurate expression of the condition of the antral and body mucosa, at least for stomachs free of ulcer or carcinoma. If focal changes were missed, there was an equal chance of picking up focal lesions in the sections examined, which, in terms of significant change, is the more serious error.

Stigmata of gastritis were searched for in each specimen and the findings were recorded separately for the antrum and body. For the purposes of this study, only the principal anatomic features of chronic gastritis were recorded; *viz.*, lymphocytic and plasma cell infiltration, lymph follicles, atrophy of the glands, metaplasia to an intestinal mucosal pattern and, additionally for the body mucosa, the pseudopyloric glands (Stoerk [5]). The degree of change for each of these features was arbitrarily graded from 1 to 3 and the specimens were divided into three groups on the basis of the following criteria:

Group 1. Antrum. The glands are regularly distributed, free from atrophy and of metaplasia to intestinal type. The stroma is scant and free, or almost free, from lymphocytes and plasma cells other than a rare basilar lymphoid aggregate with or without a germinal center (Fig. 5).

Body. The glands are regularly arranged and intact. The stroma contains no, or very few, free cells in the interfoveolar areas (Fig. 1).

Group 2. Antrum. The glands show the same features as those of group 1. The stroma shows mild lymphocytic and plasma cell infiltration (grade 1) and rare lymphoid follicles (grade 1) (Fig. 6).

Body. The glands are the same as in group 1. The stroma shows

mild superficial lymphocytic infiltration (grade 1) and not more than an occasional follicle (grade 1) (Fig. 3).

Group 3. Antrum. Here the changes are more variable. Moderate lymphocytic and plasma cell infiltration alone (grade 2), atrophy (grade 1 to 3) alone, or metaplasia (grade 1 to 3) alone was sufficient to place a case in this group. The great majority of specimens showed combinations of stromal and glandular changes (Figs. 7 and 11).

Body. Although moderate lymphocytic and plasma cell infiltration (grade 2) alone placed a case in this group, the majority showed associated glandular changes in the form of atrophy, grade 1 to 3, or metaplasia, grade 1 to 3. Moderately severe infiltration was seldom seen in the absence of glandular changes (Figs. 2, 4 and 8).

B. Surgical Material

The surgical material included in this study consisted for the most part of stomachs excised for ulcer or carcinoma. A few cases in which the excised specimen showed only benign polyps or atrophic gastritis were also included. Except for the removal of long segments from the greater curvature in 8 resections of the fundus, extensive gastric resection had been employed in all instances.

The gross specimens were routinely opened on the greater curvature, tacked to a board and floated in a 4 per cent solution of formaldehyde for fixation. Except in a few instances, fixation was begun within 10 minutes from the time the specimen was removed from the patient. In dealing with duodenal ulcer the surgical technic employed frequently left the ulcer in the inverted duodenal stump. In such cases the diagnosis of duodenal ulcer was accepted on the basis of the roentgenologic and surgical findings.

The usual histologic sections from selected areas were studied in all cases. In addition, rolled strips of mucosa from the whole length of the segments excised were prepared for section as follows: The mucosa was incised in parallel lines about 1 cm. apart and the intervening strip was separated from the muscularis propria by sharp dissection. Segments of these strips of convenient length were then rolled up, trimmed to a thickness of about 3 mm. and paraffin sections were prepared in the usual manner. In many instances segments up to 15 cm. long could be embedded in a single roll, but as many rolls were prepared as the length of the strip required. Such rolls were routinely prepared from both the greater and the lesser curvatures. In some instances additional rolls from the anterior and posterior walls were employed or these sites were substituted for the curvatures. Following resections of the fundus, rolls were prepared from the greater curva-

ture only. This method of study was apparently first employed by Hallas [6] and has been used by Hillenbrand,[4] Geissendörfer,[7] Magnus [8] and others.

The same abnormal features were noted in the surgical material as in the autopsy material (Figs. 2, 3, 4, 6, 7 and 8). For the body mucosa, "extent," indicating the proportion of the segment examined which was involved by the above changes, was graded 1 (not more than ⅓), 2 (⅓ to ⅔), or 3 (total). In addition to the three groups designated according to the several criteria as for the autopsy cases, a fourth

TABLE I

Summary of Findings, as Graded, in the Antral Mucosa of Cases Examined after Autopsy

Age	Infiltration			Follicles			Atrophy			Metaplasia			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Under 1	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	16
1–10	0	1	0	1	0	0	0	0	0	0	0	0	26	0	1	27
11–20	5	0	0	3	1	0	0	0	0	1	0	0	6	4	1	11
21–30	3	2	0	1	1	0	0	0	0	0	0	0	19	3	2	24
31–40	10	4	2	4	0	1	5	0	0	1	0	0	31	9	7	47
41–50	8	2	0	5	0	0	2	0	0	1	0	0	17	7	3	27
51–60	11	7	0	3	1	0	6	1	1	4	1	2	17	9	10	36
61–70	17	4	1	2	2	0	4	4	1	3	3	3	15	11	11	37
71–80	10	3	2	3	0	0	4	3	1	2	4	0	13	6	8	27
81–90	3	2	0	0	0	0	2	1	1	2	1	2	2	1	5	8
Totals	67	25	5	22	5	1	23	9	4	14	9	7	162	50	48	260

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

group was added for the cases with ulcer to accommodate instances in which the mucosa was hypertrophic.

GASTRITIS IN POSTMORTEM MATERIAL

For the entire group of 260 cases examined after autopsy the findings are summarized in Tables I and II for antral and body mucosa respectively. Each case was given a group designation of 1, 2, or 3 depending on the severity of the changes. Group 1 consisted of those cases which by all accepted criteria are normal. Group 2 cases were those which showed mild lymphocytic and plasma cell infiltration without parenchymal change. Although this appearance was a deviation from the usually accepted normal, it is doubtful that the degree of change exhibited is of real significance. Group 2, then, was arbitrarily designated as "almost normal." Distinctly abnormal findings placed a specimen in group 3 and these were so designated on the basis of moderate infiltration alone, or in any degree when accompanied by glandular changes. It is obvious that there is no sharp dividing line between the several degrees of lymphocytic infiltration. Thus the distinction between "mild" and "moderate" is necessarily arbitrary.

Several independent groupings of the material, however, yielded uniform results.

In respect to the antrum, 162 specimens (62.2 per cent) fell in group 1 (normal), 50 (19.2 per cent) fell in group 2 (almost normal) and 48 (18.5 per cent) were distinctly abnormal. As shown in Table I, the abnormal cases were scattered through the several decades, but increased in incidence after the age of 30 years. These findings may be compared with the findings in cases with ulcer and with carcinoma, to be discussed later, in Table IX.

TABLE II

Summary of Findings, as Graded, in the Body Mucosa of Cases Examined after Autopsy

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Under 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	16
1–10	1	1	0	4	0	0	0	0	0	0	0	0	0	0	0	25	1	1	27
11–20	2	0	0	4	0	0	0	0	0	0	0	0	0	0	0	9	2	0	11
21–30	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	19	4	1	24
31–40	9	7	1	2	1	0	1	1	0	1	0	0	1	0	0	30	9	8	47
41–50	3	2	1	2	0	0	0	0	0	0	0	0	0	0	0	21	3	3	27
51–60	3	12	4	3	1	0	3	6	3	3	3	0	6	0	1	17	3	16	36
61–70	15	4	2	1	0	0	3	3	5	6	2	2	4	2	0	16	6	15	37
71–80	2	4	4	2	0	0	2	2	2	3	1	1	2	1	0	17	2	8	27
81–90	1	3	1	0	0	0	1	1	2	0	1	1	0	1	0	3	0	5	8
Totals	40	34	13	19	2	0	10	13	12	13	7	4	13	4	1	173	30	57	260

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

For the body mucosa (Table II) 173 specimens (66 per cent) fell in group 1 (normal), 30 (12 per cent) fell in group 2 (almost normal) and 57 (22 per cent) fell in group 3 (gastritis). Of the 57 cases in group 3, all but 2 were over 30 years old, and of those which showed atrophy, all but 2 were over 50 years of age. Atrophic gastritis was present in 30 per cent of the 108 cases past 50 years of age. The findings in the body mucosa of this group may be compared with those of the surgical material in Table X.

The findings in antrum and body were not always parallel. In 13 of the specimens in which the antral mucosa was distinctly abnormal the body was normal or almost normal. These cases were scattered uniformly through the several decades. Twenty-one specimens showed a normal or almost normal antrum and a distinctly abnormal body. These appeared according to decades as follows: fourth decade, 3; fifth, 2; sixth, 8; seventh, 6; eighth, 2.

In none of the cases which revealed abnormalities was there any evidence of a causative factor such as the various exogenous and endogenous irritants to which the changes have been attributed by Faber [2] and others. For the entire group the findings were independent

of the fatal illnesses and their durations. There was no greater incidence of gastritis in individuals who died of infectious diseases. In so far as the recorded histories yielded information, there was no demonstrable relationship to previously sustained diseases. There were no sex differences. The only constant factor was that of age. Gastritis was rarely encountered below the age of 30, and of those specimens which showed atrophic changes only 2 appeared in individuals below the age of 50 years. In terms of this autopsy experience the high incidence of gastritis in adult stomachs reported by Hamperl,[3] Hillenbrand[4] and others cannot be substantiated, at least for individuals below 50 years of age. It appears that the majority of the stomachs obtained at autopsy are free of significant change.

Simple Gastritis in Relation to Gastric and Duodenal Ulcer

Since the early 1920's numerous papers have appeared emphasizing the constancy of an inflammatory process involving particularly the antrum in cases of both gastric and duodenal ulcer (Moszkowicz,[9] Konjetzny,[1,10,11] Kalima,[12] Orator,[13] Puhl,[14] Borchardt,[15] Puchert,[16] Aschner and Grossman,[17] Simpson,[18] Magnus and Rodgers[19] and others). These studies covered a large volume of material and in general the same pattern of mucosal alteration was common to all—an antral gastritis showing variably heavy lymphocytic infiltration, abnormal numbers of lymphoid follicles, atrophy and acute and chronic erosions. These same investigators reported an equally high incidence of duodenitis in their material. Changes in the body mucosa have not been as systematically investigated, particularly in respect to their topographic distribution. The reports indicate a lower incidence of gastritis involving the body mucosa in duodenal ulcer than in gastric ulcer. So-called hypertrophic gastritis is not infrequently associated with ulcer but there are few direct references to its incidence in resected material.

The findings in 106 stomachs resected for ulcer (78 duodenal, 13 gastric and duodenal, and 15 gastric) are recorded in this paper.

The antral changes rather closely paralleled those described in other reports. The gross appearance of the antral mucosa varied rather widely. Color changes were inconstant and, as congestion and areas of fresh hemorrhage dependent on surgical trauma precluded accurate observation, this feature was generally ignored. Exaggerated irregularity of the areae gastricae was not uncommon. Frequently part of the antral mucosa appeared to be more thin and smooth than normal. Such areas were most commonly seen at the apex of the antral triangle. In some instances the atrophy was diffuse but left, here and there,

islands of mucosa of more normal thickness. Grossly demonstrable erosions were infrequent. Only 2 specimens presented the typical appearance of the erosive gastritis illustrated by Konjetzny,[1] Faber,[2] Puhl [14] and others. In a few specimens one or two erosions were demonstrable.

The body mucosa regularly showed no gross changes of significance except in those instances where it could be recognized as hypertrophic.

As in the material obtained at autopsy, the histologic changes were recorded separately for antral and body mucosa. Such alterations were

TABLE III
Summary of Findings, as Graded, in the Antral Mucosa of Cases with Ulcer

Age	Infiltration			Follicles			Atrophy			Metaplasia			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
11–20	1	1	0	0	2	1	1	0	1	0	0	0	0	0	2	2
21–30	0	6	2	2	5	0	3	3	1	0	0	0	0	0	8	8
31–40	2	20	4	9	12	5	10	10	2	1	2	0	0	0	26	26
41–50	2	19	5	8	10	8	11	11	3	6	1	1	0	0	26	26
51–60	2	22	3	7	16	4	6	15	6	9	1	0	0	0	27	27
61–70	0	7	0	2	4	1	0	3	3	3	0	0	0	0	7	7
71–80	1	1	0	0	1	1	0	2	0	2	0	0	0	0	2	2
Totals	8	76	14	28	50	20	31	44	16	21	4	1	0	0	98	98

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

admirably brought out by means of the mucosal rolls inasmuch as there were often variations in distribution and severity in the antrum. The changes were quite regularly more pronounced on the lesser than on the greater curvature. The antral findings in 98 cases with ulcer are summarized in Table III. The specimens from 8 resections of the fundus, which yielded little or no antral mucosa, were excluded. As shown in the table, there were no specimens in which the antrum was normal or almost normal. All showed distinctly abnormal features and fell in group 3.

Not included in the tabulations are acute erosions which were found in but 3 specimens. Erosions were otherwise chronic, usually healed, occasionally healing. Microscopic erosions of this character were found in 25 per cent of the cases (Fig. 11).

The antral mucosa was considered to be hypertrophic in 19 specimens (not indicated in the tables). Seventeen of these were in the group with duodenal ulcers, 6 associated with hypertrophic body mucosas and 11 without. Two were found in the group with combined duodenal and gastric ulcer, 1 of which had an associated hypertrophic body mucosa. None was found in the group with gastric ulcers. The upper limit of normal thickness is not well defined. Plenk [20] considered a thickness of 1.5 mm. as normal, while Berger's [21] data indicated an

average thickness of about 0.65 mm. In my autopsy material the thicknesses in adults ranged around 0.80 to 0.90 mm. A mucosa of 1.25 mm. is thicker than any encountered in the autopsy group or in the bulk of the surgical material, although this level was approached more frequently among the surgical specimens. Most of the specimens designated as hypertrophic were 1.5 mm. or over (Fig. 10). All of these had superimposed inflammatory changes and all showed areas where destruction and atrophy had occurred. It should be emphasized that hypertrophy as it is here considered means an increase in all constituents of the mucosa so that both glands and crypts are elongated while their proportionate relationships to mucosal thickness are maintained. Relative or absolute increase in the length of the crypts at the expense of the glands proper—atrophic hyperplastic changes—are not included.

While this study is not primarily concerned with the condition of the duodenal mucosa, the findings in this series of cases may be mentioned. Inasmuch as little or no duodenal mucosa was included with many specimens, a detailed study was precluded. In some specimens in addition to true ulcer, or in the absence of an ulcer in the segment excised, distinct abnormalities were found. Lymphocytic and plasma cell infiltration exceeded normal limits and healed and healing erosions were found. No acute erosions were encountered. In some specimens the portion of the duodenum available showed no change of significance.

The frequency of abnormalities of the antral mucosa in cases with ulcer is well emphasized in Table IX, where the autopsy and surgical material may be compared. While the antral mucosa was never normal or "almost normal" among the 98 cases with ulcer, 212 of the 260 autopsy specimens were normal or almost normal. Among the 48 remaining specimens the changes were infrequently as severe as in the group with ulcer. If only the age groups of cases with ulcer are considered, the autopsy material revealed comparable degrees of gastritis in 42 of 229 cases, an incidence of 18 per cent as compared to an incidence of 100 per cent in the group with ulcer.

It may be mentioned here that attempts have been made to explain the antral findings in resected stomachs on the basis of surgical trauma. Schindler, Necheles and Gold [22] reproduced the conditions incident to gastrectomy in dogs by clamping off the stomach and tying the vessels. In parts of the stomach deprived of blood supply and exposed to acid they described alterations and hemorrhages. Their illustrations are not convincing and, when correlated with the resected human stomach, show no changes of significance.

Sanders and Mecray,[23] in a similar study, described superficial hemorrhages as gastritis and concluded that when free acid was present in the stomach the changes were proportional to the degree of vascular engorgement and to the time elapsed between resection and examination of the specimen. Their illustrations showed no evidence of gastritis and they admitted the absence of leukocytic infiltration. These authors concluded that gastroscopic examination is a better means of determining the condition of the antral mucosa than is its histologic appearance in resected stomachs. Engorgement and edema admittedly

TABLE IV

Summary of Findings, as Graded, in the Body Mucosa of Cases with Duodenal Ulcer

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Extent			Group*				Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4	
11–20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
21–30	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	7	0	0	1	8
31–40	8	1	0	6	0	0	0	0	0	0	0	0	0	0	0	0	4	5	13	2	0	9	24
41–50	9	0	0	4	2	0	1	0	0	0	0	0	0	0	0	0	5	4	10	7	1	3	21
51–60	5	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	1	4	12	5	0	0	17
61–70	4	0	0	3	0	0	1	0	0	1	0	0	1	0	0	0	2	2	2	2	1	1	6
Totals	27	1	0	19	3	0	2	0	0	1	0	0	1	0	0	0	13	15	46	16	2	14	78

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis; group 4 = cases with hypertrophy.

result from surgical manipulation. Such effects were frequently observed and as frequently ignored in the surgical material under consideration in this paper. It is inconceivable that atrophy, dense lymphocytic infiltration and healed erosions could have developed during the course of an operation even several hours long. Furthermore, entirely similar changes are found at autopsy in stomachs with ulcer when no operative trauma has complicated the picture.

The histologic findings in the body mucosa of the 106 cases of duodenal and gastric ulcer are summarized in Tables IV (duodenal ulcer), V (duodenal and gastric ulcer) and VI (gastric ulcer). Here again the mucosal rolls afforded a distinctly advantageous means of visualizing the general topography of the changes. With these resections in which 75 to 80 per cent of the stomach was routinely removed, many ordinary sections would have been required to provide the same information. Gastritic changes involving that portion of the body mucosa immediately adjacent to the transition zone were not considered. Only those where the process involved more than a segment of 1 to 2 cm. immediately adjacent to the antrum were considered abnormal.

Of the 78 resected specimens from cases of duodenal ulcer, the body

mucosa (Table IV) was without change in 46 instances (59 per cent). Sixteen cases (20.5 per cent) fell in group 2 (almost normal). Only 2 cases (2.5 per cent) fell in group 3. These, although they showed but mild infiltration, had small areas of atrophy. Fourteen cases (18 per cent) fell in group 4 (hypertrophic). In all of these the mucosa was over 1.5 mm. in thickness (Fig. 9). Four were otherwise free

TABLE V

Summary of Findings, as Graded, in the Body Mucosa of Cases with Duodenal and Gastric Ulcer

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Extent			Group*				Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4	
31–40	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1
41–50	1	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	1	1	2	0	1	1	4
51–60	0	3	0	0	2	0	1	0	0	0	0	0	0	0	0	0	1	2	1	0	3	1	5
61–70	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	3
Totals	2	5	1	3	4	0	3	0	0	0	0	0	0	0	0	1	2	5	4	1	6	2	13

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis; group 4 = cases with hypertrophy.

TABLE VI

Summary of Findings, as Graded, in the Body Mucosa of Cases with Gastric Ulcer

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseduopyloric glands			Extent			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
31–40	1	1	1	1	2	0	1	0	1	0	0	1	1	1	0	0	1	2	0	1	2	3
41–50	1	3	0	3	1	0	2	1	0	2	0	0	2	1	0	1	1	2	0	1	3	4
51–60	1	5	0	4	2	0	2	3	0	3	0	0	3	1	0	0	4	2	0	1	5	6
61–70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71–80	0	2	0	2	0	0	0	1	1	2	0	0	2	0	0	0	1	1	0	0	2	2
Totals	3	11	1	10	5	0	5	5	2	7	0	1	8	3	0	1	7	7	0	3	12	15

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

of change. Abnormalities exhibited by the remaining 10 consisted of superficial lymphocytic and plasma cell infiltration only; mild in 9, moderate in 1. In the table these features are included with those of the nonhypertrophic specimens. If the entire group is considered from the standpoint of the gastritic features only, 50 (64.1 per cent) were free of change, 25 (32 per cent) showed mild superficial lymphocytic infiltration only, 1 (1.3 per cent) showed moderate superficial infiltration only and 2 (2.6 per cent) showed in addition small areas of atrophy. The extent of the involvement was partial in 13 and total in 15.

The findings in the 13 cases with both duodenal and gastric ulcers are summarized in Table V. Four were normal, 1 was almost normal, 6 were gastritic and 2 were hypertrophic. Of the hypertrophic

specimens 1 was otherwise unchanged and 1 showed mild superficial lymphocytic infiltration.

The findings in the cases of gastric ulcer are summarized in Table VI. Among these none were normal, 3 were almost normal and 12 showed gastritis. All 12 showed at least some degree of atrophy.

Because of the differences in the findings in the three groups of cases with ulcer the data have not been consolidated. Each group may best be considered in relation to the autopsy material (Table X). Excluding instances of hypertrophy the character of the changes found in cases with ulcer and without ulcer is the same.

Those specimens in the group with duodenal ulcer in which the mucosa was abnormal showed the same age distribution as did those of the autopsy series. The total number of cases per decade was small and the numbers in the two groups were not strictly comparable. However, there appeared to be a greater incidence of mild infiltration in the group with ulcer for each decade concerned. On the other hand, severe alterations—atrophic mucosas—were much less frequently seen. In the group with ulcer there were 44 cases in the fifth to seventh decades inclusive and only 2 of these showed atrophic changes, both mild. Among the 100 autopsy cases for the same period there were 23 instances of atrophic gastritis. The distribution of the changes could not, of course, be compared. It may be reasonably concluded that in spite of the constant finding of an antral gastritis in cases of duodenal ulcer, severe changes in the body mucosa only exceptionally appear and are less numerous than might be expected in an equivalent number of individuals free of ulcer in the older age groups.

Cases in which both gastric and duodenal ulcers were present were intermediate in position between the groups with duodenal and with gastric ulcer as to changes in the body mucosa. The data may be compared with those from autopsy material in Table X, but the small number of cases precludes any conclusions.

In the group with gastric ulcer there were no specimens in which the body mucosa was entirely without change and 12 of the 15 showed at least some areas of atrophy. The cases were rather evenly distributed through the several decades and the incidence of atrophy was high in each. That atrophic changes are to be expected in cases of gastric ulcer is suggested. However, with so few cases the remote possibility of a coincidental high incidence cannot be excluded.

In this group of 106 cases with ulcer the changes were independent of all considerations other than their association with ulcer. The great majority of these patients came to operation only after years of medical management which had given no constant relief. A few had

had previous gastroenterostomies and some had had one or more hemorrhages. Many of them had high fasting acids and copious secretion. Many of them had, or at some time had exhibited, symptoms or roentgenologic evidence of obstruction. As far as the antral changes were concerned there was no relation to the character or duration of symptoms or to the exhibited secretory activity. The mucosal changes were as severe in a boy of 13 years whose symptoms covered a 3-year period as they were in much older individuals who reported periodic episodes of distress of 10 or even 20 years' duration. Patients in whom the body mucosa was found to be hypertrophic showed no greater acidity or more severe symptoms than did those in whom the mucosa was of normal thickness. Changes in neither the antrum nor the body were in any way related to obstruction. The body mucosa was found to be entirely normal in cases of long-standing, high-grade obstruction, while in others, in which no obstruction had occurred, there was a superficial gastritis. The cases of gastric ulcer in general showed lower acidity than did the group with duodenal ulcer. Perhaps this finding is related to the higher incidence of atrophic gastritis in the cases of gastric ulcer, although the case which presented the most extensive atrophic changes had normal acid values. There was no relationship between the findings and the size of the ulcer or the presence or absence of perforation. In some cases very small ulcers were found which were hardly consistent with the duration of symptoms. The cases in which the resected specimens showed acute erosions and those with a grossly demonstrable erosive gastritis had no unusual clinical features.

Discussion of Gastritis Associated with Ulcer

The findings in this series of cases support the frequently reported constancy of a gastritis associated with ulcer. There is good evidence that the process is primary and not secondary to the ulcer. The frequent antrum-wide distribution is by itself evidence for its primary character. Additional evidence is also to be gained from the not infrequent instances in which stomachs resected for typical symptoms of ulcer have failed to reveal ulcers but have shown an erosive gastritis and duodenitis (Puhl,[14] Aschner and Grossman,[17] Konjetzny,[24] Faber,[25] Paaby,[26] Nicholaysen,[27] Dahl,[28] Holsti,[29] Roholm [30] and Andersen [31]).

It appears, then, that true ulcer is superimposed on the gastritis (and duodenitis) to the erosive lesions of which Konjetzny,[1] Puhl [14] and others traced its origin. In the chronic changes they saw the healed and healing stages of repeated acute inflammatory attacks. Puhl considered that periodicity of symptoms presented by patients with

ulcer found anatomic expression in the simultaneous presence of acute erosions and their healing stages in resected specimens. Further evidence for this concept was provided by the stomachs of calves in which the appearance of ulcers following weaning had been long known. In these animals Konjetzny and Puhl [32] found acute and chronic inflammatory changes and all transitions from erosions to true ulcers. In my own human material the changes are so predominantly chronic that an evolution from acute inflammatory attacks cannot be traced. It has been suggested that longer and more adequate preoperative medical management accounts for the infrequency of acute lesions in the more recent material (Aschner and Grossman [17]). Puhl's [14] case histories, for example, reveal that operative interference was employed at the height of acute symptoms in many instances.

Experimental evidence for a gastritic basis for chronic ulcer is seen in the cinchophen ulcers produced in dogs (Van Wagoner and Churchill,[33] Bollman, Stalker and Mann [34] and Simonds [35]) and cats (Schwartz and Simonds [36]) where an acute diffuse gastritis with multiple erosions precedes the larger lesion.

Other recent experimental work has emphasized the importance of the acid factor in ulcer genesis. Although numbers of investigators have reported superficial erosions as the result of elevated gastric secretion in animals (Büchner, Siebert and Molloy,[37] Simpson,[38] Overgaard [39] and others), no lesions similar to the chronic ulcer of man were produced. Employing Code and Varco's [40] method of daily injections of histamine-base in beeswax for inducing prolonged hypersecretion, Walpole [41] and Hay [42] with their collaborators have produced true chronic ulcers in cats, dogs and other animals. The changes preceding the appearance of the ulcers have not been described. Some of their specimens which I have been privileged to examine showed no significant mucosal alterations near to, or at a distance from, the ulcers. If anything resembling a diffuse gastritis preceded the appearance of actual ulceration, it left no traces. Whether such changes may have occurred or whether the acidity attained in these animals resulted in a primary cauterization of the mucosa is not known.

While acid must be admitted as a constant factor in the problem of "peptic" ulcer, the antral gastritis is also constant. Its predominant limitation to the antrum indicates that it is a special form of gastritis which must be separated from the process leading to atrophy and achlorhydria where the body is regularly involved. If acid is to be considered the prime factor in the causation of ulcer, it must also be responsible for the accompanying gastritis. That acid secretion anywhere within possible physiological limits can initiate such a process

seems doubtful. While ulcer patients often secrete copious amounts of highly acid gastric juice, whether such secretory activity prevailed prior to the development of ulceration is not known. Faber [2] considered this hypersecretion to be largely accounted for by the pathologic aftersecretion resulting from the irritating antral gastritis. While it is not theoretically impossible that acid may cause or be a factor in the causation of a gastroduodenitis, the gaps between the condition in man and the acid production of ulcer in animals are wide. That acid produces the constantly observed changes in the antral mucosa requires demonstration.

No conclusive evidence pointing to an etiologic factor for the gastritis with ulcer has been produced. Konjetzny [1] emphasized the importance of a variety of exogenous irritants. In this connection he attributed the gastritis of calves to the coarse foods which supplanted milk at the time of weaning. That certain foods may under certain circumstances have a deleterious effect has been suggested by experimental observations. Bollman, Stalker and Mann [34] found that coarse foods decreased and soft foods increased the time required for the appearance of cinchophen ulcers in dogs. Simpson [38] was able to produce erosions in his animals with sustained high acid values only when mustard oil was added to their food. However, the importance of food irritants as a factor in the production of this form of gastritis in man is as difficult to substantiate as it is to consider them of importance in the evolution of atrophic pangastritis.

Irrespective of the primary basis of the antral gastritis there is no doubt that the changes constantly accompany gastric ulcer, and Konjetzny's [24] original dictum that an ulcer does not develop in a healthy mucosa still holds for the majority of the cases.

Simple Gastritis in Relation to Carcinoma of the Stomach

The frequent association of widespread gastritis and carcinoma of the stomach has long been known, and has been discussed by many authors on the basis of both autopsy and surgical material (Geissendörfer,[7] Borchardt,[15] Puchert,[16] Simpson,[18] Mathieu,[43] Saltzman,[44] Konjetzny,[1, 45, 46] Orator,[47] Judd [48] and others). The diffuse distribution of these mucosal alterations rather sharply distinguishes the process from that associated with ulcer. Several authors have commented upon the differences. Konjetzny,[1] Orator,[13] Borchardt [15] and others have emphasized the fact that the gastritis of ulcer is antrum-confined for the most part while that of cancer is a pangastritis. Although this point remains the chief distinguishing feature, other

dissimilarities have been noted. Intestinal metaplasia is largely focal with ulcer and often diffuse with cancer (Geissendörfer,[7] Moszkowicz,[9] Kalima,[12] Borchardt [15]). Larger numbers of Russell's corpuscles are seen in stomachs with cancer than in those with ulcer (Konjetzny,[1] Kalima,[12] Puchert [16]). Konjetzny [1] claimed that the gastritis associated with ulcer showed more evidence of hyperplasia and less outspoken atrophy. Largely on this basis Konjetzny considered the gastritis with ulcer as a subacute process in contrast to the chronic gastritis with carcinoma. All of these changes are, however, subject to variation and no constant distinctions exist for the single case.

Of particular interest in the matter of gastritis associated with carcinoma is the question of its relationship to the tumor. Most of the early writers considered the gastritis to be independent of, or in some way secondary to, the tumor (Rosenheim,[49] Matti [50]). Although Mathieu [43] had early suggested the origin of carcinoma on the basis of a pre-existing gastritic mucosa, it was not until Saltzman [44] and Konjetzny [45] independently presented their material that the gastritic basis for carcinoma was seriously considered. Konjetzny [1, 45, 46] saw in the abnormal reparative processes of gastritis, particularly adenomatous polypoid overgrowth, all transitions to frank carcinoma. He claimed a demonstrable relationship to the parenchymal changes of chronic gastritis for 90 per cent of carcinomas, and further pointed out that the early carcinomas described by Hauser, Versé and others presented a similar relationship. That a carcinoma develops more commonly in stomachs which show chronic atrophic gastritis has come to be rather generally accepted (Orator,[13] Hurst [51]). Several authors have commented upon the coincidence of pernicious anemia and carcinoma of the stomach and have maintained that atrophic gastritis is the common link (Miller,[52] Jenner [53] and others).

There have been objections to the concept established by Saltzman [44] and Konjetzny.[45] Borrmann,[54] in particular, questioned the interpretation of the histologic findings of those authors, and doubted that the changes they described necessarily preceded the carcinoma. He believed that the "gastritis to gastritis polyposa to carcinoma" sequence stressed by Konjetzny [45, 46] was very much the exception, and concluded that most carcinomas arose without, or independent of, preexisting inflammatory changes. Wanser [55] rightfully emphasized the benign character of the frequently long-standing chronic gastritis associated with ulcer and doubted the existence of a causal relationship between such mucosal changes and carcinoma.

The mucosal findings in the resected specimens from 52 cases of carcinoma of the stomach are reported here. Only those specimens

which afforded relatively large amounts of mucosa uninvolved by tumor were included. Because the tumor most frequently occupied the antrum, the findings in the antral mucosa were recorded in but 27 cases. Similar circumstances excluded the body in 2 cases.

The antral findings are summarized in Table VII. There were no cases in which the antral mucosa was normal although in most the changes were relatively mild. In Table IX the changes may be compared as to severity with those associated with ulcer and in material obtained postmortem.

TABLE VII

Summary of Findings, as Graded, in the Antral Mucosa of Cases with Carcinoma of the Stomach

Age	Infiltration			Follicles			Atrophy			Metaplasia			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
31–40	1	1	0	2	0	0	1	1	0	0	1	0	0	0	2	2
41–50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51–60	5	2	0	5	0	0	5	0	1	2	0	1	0	1	6	7
61–70	9	4	0	7	2	0	6	2	3	4	2	3	0	2	11	13
71–80	3	2	0	3	1	0	4	0	1	1	1	1	0	0	5	5
Totals	18	9	0	17	3	0	16	3	5	7	4	5	0	3	24	27

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

TABLE VIII

Summary of Findings, as Graded, in the Body Mucosa of Cases with Carcinoma of the Stomach

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Extent			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
31–40	0	2	0	2	0	0	1	1	0	1	0	0	2	0	0	0	1	1	0	0	2	2
41–50	0	3	0	2	1	0	0	0	3	1	1	0	0	3	0	0	0	3	0	0	3	3
51–60	5	6	1	8	2	0	0	3	9	4	1	7	7	2	2	1	2	9	1	0	12	13
61–70	5	10	3	11	5	0	0	2	13	3	4	6	7	6	2	2	6	10	2	2	16	20
71–80	7	4	0	6	1	0	0	3	6	1	1	4	6	3	0	3	2	6	1	2	9	12
Totals	17	25	4	29	9	0	1	9	31	10	7	17	22	14	4	6	11	29	4	4	42	50

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

The findings in the body mucosa are summarized in Table VIII. Four were normal, 4 almost normal and 42 distinctly abnormal. Of the 42, 41 showed atrophic changes in at least some degree and extent. These findings may be compared with those of the ulcer and autopsy groups in Table X. In this table the cancer cases showing gastritis with or without atrophy appear in one column and those with atrophy in another.

DISCUSSION OF GASTRITIS ASSOCIATED WITH CARCINOMA

That gastritis associated with carcinoma is a pangastritis in contrast to the antral gastritis of stomachs with ulcer has been noted. There

is no direct evidence as to the time required for the development of an atrophic gastritis, but if carcinomas are prone to develop in stomachs which show an atrophic gastritis such as is commonly seen in pernicious anemia, the changes should, in the majority of cases, be extensive and severe. It is of interest to compare the mucosal changes in this series of stomachs with carcinoma with those found in a group of noncancerous stomachs in which atrophic gastritis was present. For this purpose 7 resected stomachs were available, some of which showed benign polyps and others only an atrophic gastritis.

TABLE IX

Findings in Antral Mucosa in Control Autopsy Material and in Cases of Ulcer and Cancer of the Stomach

Age	Autopsy material				With ulcer				With carcinoma			
	Total	Normal	Almost normal	Gastritis	Total	Normal	Almost normal	Gastritis	Total	Normal	Almost normal	Gastritis
Under 1	16	16	0	0	..	..	..	..	..	..	..	..
1–10	27	26	0	1	..	..	..	..	..	..	..	..
11–20	11	6	4	1	2	0	0	2	..	..	..	..
21–30	24	19	3	2	8	0	0	8	..	..	..	..
31–40	47	31	9	7	26	0	0	26	2	0	0	2
41–50	27	17	7	3	26	0	0	26	0	0	0	0
51–60	36	17	9	10	27	0	0	27	7	0	1	6
61–70	27	15	11	11	7	0	0	7	13	0	2	11
71–80	27	13	6	8	2	0	0	2	5	0	0	5
81–90	8	2	1	5	..	..	..	..	..	..	..	..
Totals	260	162	50	48	98	0	0	98	27	0	3	24

The resections were as high as in the other group so the mucosa could be examined at equivalent levels. The antral changes were of the same character as those found in the carcinoma group. While the number of cases was small, the changes appeared to be of the same magnitude. The body mucosa was severely affected in all 7 cases. Each specimen showed an advanced atrophic gastritis in which very few if any specific glands remained. These findings are comparable to those in many of the cancer group and are strikingly similar to cases where the carcinoma developed in individuals with pernicious anemia. The similarity is much less marked in other cases. Referring to Table VIII, the extent of involvement in the 50 body mucosas was: none in 4 cases, grade 1 in 6 cases, grade 2 in 11 cases and grade 3 in 29 cases. In those where extent was graded 1 and 2, that portion of the mucosa adjacent to the tumor—the lower body for the most part—showed changes, while the upper one-half or one-third of the segment was free of significant change. Thus there were all transitions, from mucosas completely normal to those where the whole segment

TABLE X

Findings in Body Mucosa in Material Obtained by Autopsy and in Cases of Ulcer and of Cancer of the Stomach

Age	Autopsy material					With duodenal ulcer						With duodenal and gastric ulcer						With gastric ulcer						With carcinoma					
	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Hypertrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Hypertrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy		Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	
Under 1	16	16	0	0	0																								
1–10	27	25	1	0	0																								
11–20	11	9	2	0	0	2	2	0	0	0	0																		
21–30	24	19	4	1	0	8	7	0	0	0	1																		
31–40	47	30	9	8	2	24	13	2	0	0	9	1	0	0	1	0	0	3	0	1	2	2		2	0	0	2	2	
41–50	27	21	3	3	0	21	10	7	1	1	3	4	2	0	1	1	1	4	0	1	3	3		3	0	0	3	3	
51–60	36	17	3	16	12	17	12	5	0	0	0	5	1	0	3	1	1	6	0	1	5	5		13	1	0	12	12	
61–70	37	16	6	15	11	6	2	2	1	1	1	3	1	1	1	1	0							20	2	2	16	15	
71–80	27	17	2	8	6													2	0	0	2	2		12	1	2	9	9	
81–90	8	3	0	5	4																								
Totals	260	173	30	57	35	78	46	16	2	2	14	13	4	1	6	3	2	15	0	3	12	12		50	4	4	42	41	

was abnormal. Moreover, variable degrees of extent as to severity of the process existed within the group graded 3. As already indicated, many of these specimens showed changes identical with those found in the noncancerous stomachs. In an appreciable number, however, the following appearances were presented:

a. Lower body adjacent to the tumor: Thin mucosa exhibiting complete loss of specific glands, more or less metaplasia to an intestinal type and variable lymphocytic infiltration.

b. Midportion of segment: Mucosa of normal thickness, some specific glands retained, while others showed deepened, regularly arranged pits lined in part by intestinal epithelium, some pseudopyloric glands and variable degrees of lymphocytic infiltration.

c. In upper part of segment: Mucosa of normal thickness with mild to moderate superficial lymphocytic infiltration but glands retained. An appearance such as that just described was by no means as sharply defined as this outline indicates, but that general pattern was presented and even in those cases where destruction and disorganization of the mucosa involved the higher reaches of the segment the process was frequently less pronounced near the plane of excision.

In one specimen in which the tumor was situated at the cardia, the changes presented themselves in the reverse manner. The most pronounced gastritic alterations were at the upper end of a segment from the greater curvature, and the mucosa became almost normal as the antrum was approached.

The apparent manner of development and progression to atrophy of the process just described and the presence in this series of several completely normal and other only partially involved body mucosas indicated that carcinoma in a distinctly appreciable number of cases was not preceded by a diffuse atrophic gastritis. In general, the most severe atrophy was associated with the larger tumors. Yet of the 4 cases in which the body mucosa was normal, 3 had relatively large antral tumors. There was no correlation between the degree of atrophic gastritis and the severity or duration of obstruction. There was no evidence that any carcinoma in this series had developed on the basis of a pre-existing ulcer. (Some authors have indicated that in stomachs with cancer but without diffuse gastritis the carcinoma is a malignant change in an ulcer: Borchardt,[15] Puchert,[16] Orator,[47]).

That the atrophic process may develop under the influence of the tumor is suggested by the findings detailed above. Particularly is that true in the case of the tumor at the cardia, where, contrary to the usual pattern of an ascending atrophy, the atrophy proceeded from above downward. Yet one cannot eliminate the possibility that some

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	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Hypertrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Hypertrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy
Under 1	16	16	0	0	0																						
1–10	27	25	1	1	0																						
11–20	11	9	2	0	0	2	2	0	0	0	0																
21–30	24	19	4	1	0	8	7	0	0	0	1																
31–40	47	30	9	8	2	24	13	2	0	0	9	1	0	0	1	0	0	3	0	1	2	2	2	0	0	2	2
41–50	27	21	3	3	0	21	10	7	1	1	3	4	2	0	1	1	1	4	0	1	3	3	3	0	0	3	3
51–60	36	17	3	16	12	17	12	5	0	0	0	5	1	0	3	1	1	6	0	1	5	5	13	1	0	12	12
61–70	37	16	6	15	11	6	2	2	1	1	1	3	1	1	1	1	0						20	2	2	16	15
71–80	27	17	2	8	6													2	0	0	2	2	12	1	2	9	9
81–90	8	3	0	5	4																						
Totals	260	173	30	57	35	78	46	16	2	2	14	13	4	1	6	3	2	15	0	3	12	12	50	4	4	42	41

was abnormal. Moreover, variable degrees of extent as to severity of the process existed within the group graded 3. As already indicated, many of these specimens showed changes identical with those found in the noncancerous stomachs. In an appreciable number, however, the following appearances were presented:

a. Lower body adjacent to the tumor: Thin mucosa exhibiting complete loss of specific glands, more or less metaplasia to an intestinal type and variable lymphocytic infiltration.

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That the atrophic process may develop under the influence of the tumor is suggested by the findings detailed above. Particularly is that true in the case of the tumor at the cardia, where, contrary to the usual pattern of an ascending atrophy, the atrophy proceeded from above downward. Yet one cannot eliminate the possibility that some

of these cases are examples of the coincidental relationship of a developing atrophic gastritis, which is not uncommon in the age periods concerned, and a carcinoma. This explanation is plausible, but in the absence of known etiologic factors for an atrophic gastritis, no definite conclusions are justified.

That an appreciable number of these carcinomas apparently developed in stomachs which were not the seat of a long-standing atrophic gastritis affects but little the question of the primary genesis of the tumor. The tumors were, for the most part, large and beyond the stage at which evidence for their early development could be found. It is true that, particularly among the tumors associated with a preexisting diffuse atrophic gastritis, there was often at the growing margins of the tumor a blending of the carcinoma with the abnormal glands. In some specimens there were multiple foci of origin in an atrophic, metaplastic mucosa beyond the main body of the tumor. In a few specimens there were transitions between polypoid overgrowths and carcinoma. In other instances the mucosa in the immediate vicinity of relatively large carcinomas showed little change. That finding alone cannot eliminate the possibility that the tumor concerned arose in a localized abnormal area in the mucosa. One may agree with Borrmann [54] that carcinomas developing on the basis of a gastritis polyposa are the exception rather than the rule. The earliest carcinomas in this series were flat, ulcerating lesions which, on a somewhat larger scale, followed the ulcerative changes noted by Mallory [56] in his cases of carcinoma *in situ*.

Summary and Conclusions

The manifestations of chronic gastritis encountered in 260 stomachs obtained at autopsy, in 106 stomachs resected for duodenal or gastric ulcer and in 52 stomachs resected for carcinoma are reported.

The autopsy series included individuals of all ages, dying from a variety of causes, whose histories revealed no evidence of gastric symptoms. The changes exhibited by this group were chiefly those of a pangastritis leading to atrophy. The process bore no demonstrable relationship to any factor other than age. Gastritic changes in any degree were rare below the age of 30 years and severe changes were uncommon below the age of 50. Atrophic gastritis was exhibited by 30 per cent of the 108 cases past 50 years of age. This form of gastritis is unimportant in resected stomachs from individuals under 50 years of age.

Among the 106 stomachs resected for ulcer (78 duodenal, 13 gastric and duodenal, 15 gastric) there was an antral gastritis in all of the

98 cases in which the antrum was examined. Changes in the body mucosa were rare in the duodenal ulcer group and common in the gastric ulcer group. The evidence substantiates the contention that an antral gastritis (and duodenitis) precedes, and is the anatomic basis for, the development of chronic ulcer.

The gastritic changes encountered in 52 resected carcinoma-bearing stomachs were variable in extent and severity. Although numbers of these stomachs presented a diffuse atrophic gastritis which undoubtedly antedated the tumors, that condition did not obtain for all. In a few instances the body mucosa was normal or nearly normal. In others, although there were severe changes in the vicinity of the tumor, the more distant portions of the excised segments were less, or not at all, affected. The findings in such cases suggested that the mucosal changes were secondary to the tumor, although the possibility of a coincidental association of the tumor and a developing atrophic gastritis could not be excluded. There was in this series no evidence to indicate that carcinomas arise with unusual frequency in stomachs already the seat of a diffuse atrophic gastritis.

REFERENCES

1. Konjetzny, G. E. Die Entzündungen des Magens. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1928, **4**, pt. 2, 768–1116.
2. Faber, Knud. Gastritis and Its Consequences. G. Forlagstrykkeri, Copenhagen, 1935.
3. Hamperl, H. Ueber akute Gastritis. *Wien. klin. Wchnschr.*, 1932, **45**, 513–514.
4. Hillenbrand, Karl. Histotopographische und histologische Untersuchungen über die sog. chronische Gastritis. *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, **85**, 1–32.
5. Stoerk, Oskar. Ueber gastritis chronica. *Wien. klin. Wchnschr.*, 1922, **35**, 855–860.
6. Hallas, E. A. Über heterotope Epithelproliferationen bei Gastritis chronica. *Virchows Arch. f. path. Anat.*, 1911, **206**, 272–278.
7. Geissendörfer, Rudolf. Untersuchungen über Vorkommen, Lokalisation und Ausbreitungsweise der Umbaugastritis in Carcinommägen. *Arch. f. klin. Chir.*, 1928, **153**, 235–252.
8. Magnus, H. A. Observations on the presence of intestinal epithelium in the gastric mucosa. *J. Path. & Bact.*, 1937, **44**, 389–398.
9. Moszkowicz, Ludwig. Zur Histologie des ulcusbereiten Magens. *Arch. f. klin. Chir.*, 1923, **122**, 444–499.
10. Konjetzny, G. E. Chronische Gastritis und Duodenitis als Ursache des Magenduodenalgeschwürs. *Beitr. z. path. Anat. u. z. allg. Path.*, 1923, **71**, 595–618.
11. Konjetzny, G. E. III. Die entzündliche Grundlage der typischen Geschwürsbildung im Magen und Duodenum. *Ergebn. d. inn. Med. u. Kinderh.*, 1930, **37**, 184–332.
12. Kalima, Tauno. Pathologisch-anatomische Studien über die Gastritis des Ulcusmagens nebst einigen Bemerkungen zur Pathogenese und pathologischen Anatomie des Magengeschwürs. *Arch. f. klin. Chir.*, 1924, **128**, 20–108.

13. Orator, V. Beiträge zur Magenpathologie (histologische Untersuchungen an klinischem Resektionsmaterial). I. Das Magen-Duodenal- und postoperative Jejunalgeschwür. *Virchows Arch. f. path. Anat.*, 1925, **255**, 639–676.
14. Puhl, Hugo. Über die Bedeutung entzündlicher Prozesse für die Entstehung des Ulcus ventriculi et duodeni. *Virchows Arch. f. path. Anat.*, 1926, **260**, 1–109.
15. Borchardt, Harold. Über das Verhalten der Magenschleimhaut beim Carcinoma ventriculi, beim Ulcus ventriculi und beim Carcinoma ex ulcere. *Virchows Arch. f. path. Anat.*, 1929, **275**, 790–811.
16. Puchert, Horst. Über die Magenschleimhaut bei Geschwür und bei Krebs, mit Berücksichtigung des lymphatischen Gewebes. *Virchows Arch. f. path. Anat.*, 1931, **280**, 385–404.
17. Aschner, P. W., and Grossman, Sidney. Gastritis and duodenitis in relation to the ulcer problem. A study of 124 cases of partial gastrectomy. *Surg., Gynec. & Obst.*, 1933, **57**, 334–342.
18. Simpson, C. K. Observations upon gastritis. *Guy's Hosp. Rep.*, 1935, **85**, 102–125.
19. Magnus, H. A., and Rodgers, H. W. The mucosa of the body of the stomach in chronic gastroduodenal ulceration. *St. Barth. Hosp. Rep.*, 1938, **71**, 129–140.
20. Plenk, Hanns. Der Magen. In: von Möllendorf, Wilhelm. Handbuch der mikroskopischen Anatomie des Menschen. J. Springer, Berlin, 1932, **5**, pt. 2, 1–234.
21. Berger, E. H. The distribution of parietal cells in the stomach, a histotopographic study. *Am. J. Anat.*, 1934, **54**, 87–114.
22. Schindler, R.; Necheles, H., and Gold, R. L. Surgical gastritis. A study of the genesis of gastritis found in resected stomachs with particular reference to the so-called "antral gastritis" associated with ulcer. *Surg., Gynec. & Obst.*, 1939, **69**, 281–286.
23. Sanders, G. B., and Mecray, P. M. Pseudogastritis of operative origin. *Ann. Surg.*, 1941, **114**, 986–996.
24. Konjetzny, G. E. Zur chirurgischen Beurteilung der chronischen Gastritis. *Arch. f. klin. Chir.*, 1924, **129**, 139–171.
25. Faber, Knud. The pyloric symptom complex. *Acta med. Scandinav.*, 1928, suppl. **26**, 358–362.
26. Paaby, H. Pylorusgastritis. *Acta med. Scandinav.*, 1928, suppl. **26**, 363–371.
27. Nicolaysen, Johan. Chronic gastritis regarded from a surgical standpoint. *Acta chir. Scandinav.*, 1928, **63**, 87–105.
28. Dahl, Bjarne. Une étude anatomo-pathologique et clinique sur la gastrite aigue et chronique. *Arch. d. mal. de l'app. digestif.*, 1930, **20**, 761–807.
29. Holsti, Osten. On the nature of the pylorus-affections, which simulate ulcus. *Acta med. Scandinav.*, 1931, **76**, 343–393.
30. Roholm, Kaj. Über den Wert der zirkulären Resektion bei ulcus chronicum ventriculi s. duodeni. *Acta chir. Scandinav.*, 1933–34, **73**, 433–484.
31. Andersen, Torben. Über gastroduodenitis. *Acta med. Scandinav.*, 1934–35, **84**, 185-216.
32. Konjetzny, G. E., and Puhl, H. Das sogenannte Ulcus pepticum des Magens der Absatzkälber. *Virchows Arch. f. path. Anat.*, 1926, **262**, 615–633.
33. Van Wagoner, F. H., and Churchill, T. P. Production of gastric and duodenal ulcers in experimental cinchophen poisoning of dogs. *Arch. Path.*, 1932, **14**, 860–869.
34. Bollman, J. L.; Stalker, L. K., and Mann, F. C. Experimental peptic ulcer produced by cinchophen. *Arch. Int. Med.*, 1938, **61**, 119–127.
35. Simonds, J. P. Mode of origin of experimental gastric ulcer induced by cinchophen. *Arch. Path.*, 1938, **26**, 44–50.

36. Schwartz, S. O., and Simonds, J. P. Peptic ulcers produced by feeding cinchophen to mammals other than the dog. *Proc. Soc. Exper. Biol. & Med.*, 1934–35, **32,** 1133–1134.
37. Büchner, F.; Siebert, P., and Molloy, P. J. Über experimentell erzeugte akute peptische Geschwüre des Rattenvormagens. *Beitr. z. path. Anat. u. z. allg. Path.*, 1928–29, **81,** 391–425.
38. Simpson, C. K. Observations on gastritis. *Guy's Hosp. Rep.*, 1934, **84,** 351–362.
39. Overgaard, Kristian. Experimentelle Untersuchungen über die Entwickelung der Antrumgastrit. *Acta med. Scandinav.*, 1934, **81,** 429–486.
40. Code, C. F., and Varco, R. L. Chronic histamine action. *Proc. Soc. Exper. Biol. & Med.*, 1940, **44,** 475–477.
41. Walpole, S. H.; Varco, R. L.; Code, C. F., and Wangensteen, O. H. Production of gastric and duodenal ulcers in the cat by intramuscular implantation of histamine. *Proc. Soc. Exper. Biol & Med.*, 1940, **44,** 619–621.
42. Hay, L.; Varco, R. L.; Code, C. F., and Wangensteen, O. H. Personal communication from Dr. Hay.
43. Mathieu, Albert. État de la muqueuse de l'estomac dans le cancer de cet organe. *Arch. gén. de méd.*, 1889, **1,** 402–420; 571–588.
44. Saltzman. Cited by Konjetzny.[1,46]
45. Konjetzny, G. E. Ueber die Beziehungen der chronischen Gastritis mit ihren Folge-erscheinungen und des chronischen Magenulcus zur Entwicklung des Magenkrebses. *Beitr. z. klin. Chir.*, 1913, **85,** 455–519.
46. Konjetzny, G. E. Allgemeine Pathologie und spezielle pathologische Anatomie. In: Anschütz, Wilhelm and Konjetzny, G. E. Die Geschwülste des Magens. F. Enke, Stuttgart, 1921, pt. 1.
47. Orator, V. Beiträge zur Magenpathologie IV. *Arch. f. klin. Chir.*, 1925, **134,** 663–681.
48. Judd, E. S. Possible relationship of residual lesions of ulcerative gastritis to the development of carcinoma of the stomach. *Proc. Staff Meet., Mayo Clin.*, 1939, **14,** 52–56.
49. Rosenheim, Theodor. Ueber atrophische Processe an der Magenschleimhaut in ihrer Beziehung zum Carcinom und als selbständige Erkrankung. *Berl. klin. Wchnschr.*, 1888, **25,** 1021–1025.
50. Matti, Hermann. Beitrag zur Kenntnis des Magencarcinoms. Untersuchungen über die Ursachen des veränderten Chemismus bei Fallen von Magenkrebs. *Deutsche Ztschr. f. Chir.*, 1910, **104,** 425–506.
51. Hurst, A. F. Precursors of carcinoma of the stomach. *Lancet*, 1929, **2,** 1023–1028.
52. Miller, T. G. Addisonian anemia and carcinoma of stomach in the same individual. Report of three cases: Chronic gastritis as a probable basis for both diseases. *Internat. Clin.*, 1935, s. 45, **1,** 167–181.
53. Jenner, A. W. F. Perniziöse Anämie und Magenkarzinom. *Acta med. Scandinav.*, 1939, **102,** 529–590.
54. Borrmann, R. Geschwülste des Magens und Duodenums. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1926, **4,** pt. 1, 897–902.
55. Wanser, R. Die banale chronische Gastritis und ihre Beziehungen zum Magenkarzinom. *Beitr. z. path. Anat. u. z. allg. Path.*, 1939, **103,** 113–156.
56. Mallory, T. B. Carcinoma *in situ* of the stomach and its bearing on the histogenesis of malignant ulcers. *Arch. Path.*, 1940, **30,** 348–362.

DESCRIPTION OF PLATES

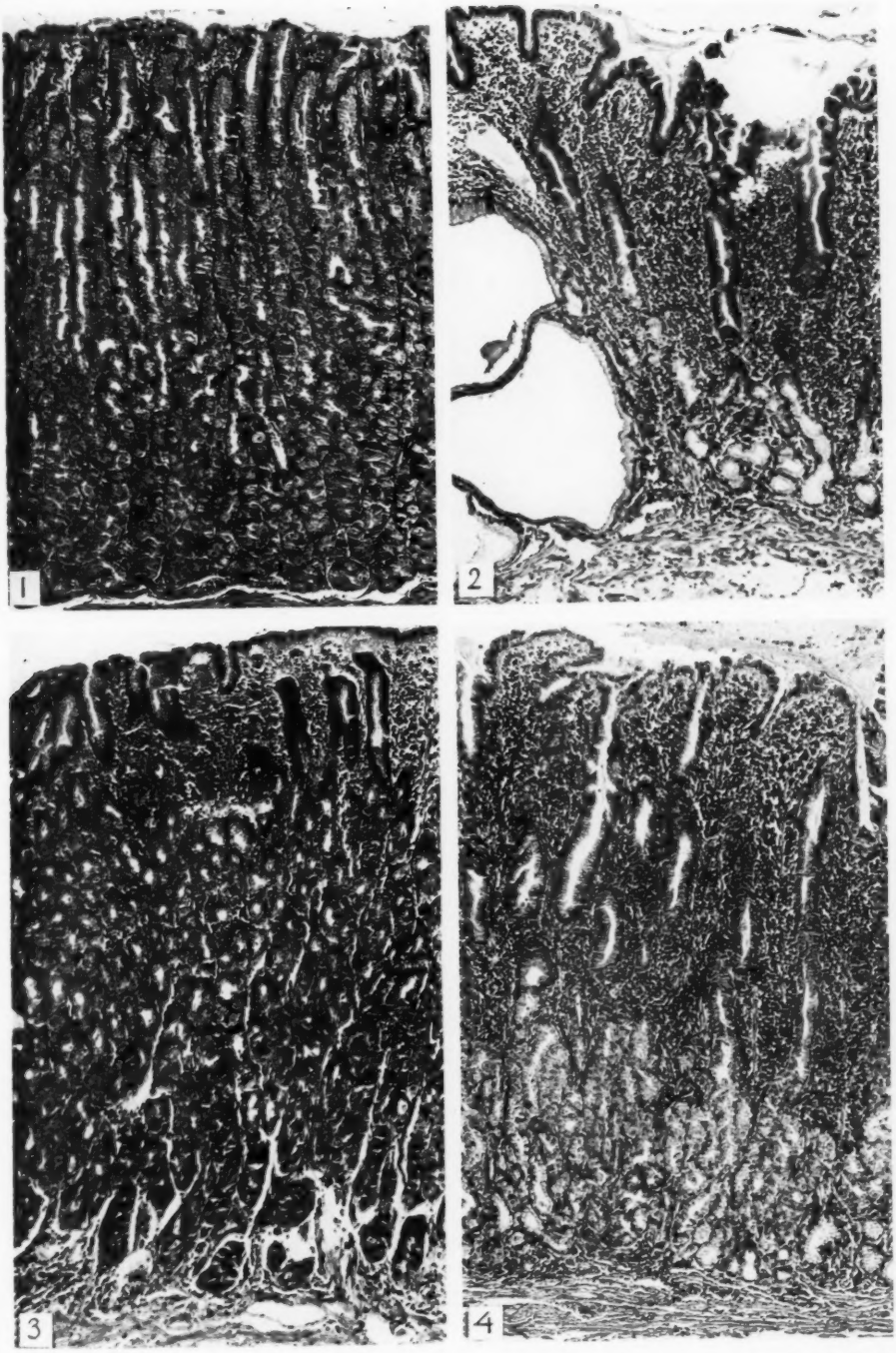
PLATE 7

FIG. 1. Normal body mucosa. Hematoxylin and eosin stain. × 75.

FIG. 2. Body mucosa showing advanced atrophy, cystic and pseudopyloric glands and moderate lymphocytic infiltration. Hematoxylin and eosin stain. × 75.

FIG. 3. Body mucosa showing mild superficial lymphocytic infiltration. The glands are intact. Hematoxylin and eosin stain. × 75.

FIG. 4. Body mucosa showing moderate lymphocytic infiltration and moderate atrophy. There are deepened pits. The basilar portions of the glands are well retained. Hematoxylin and eosin stain. × 75.



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Chronic Gastritis

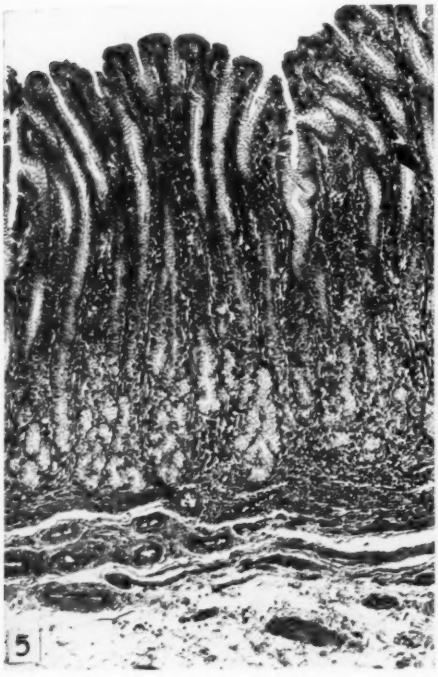
PLATE 8

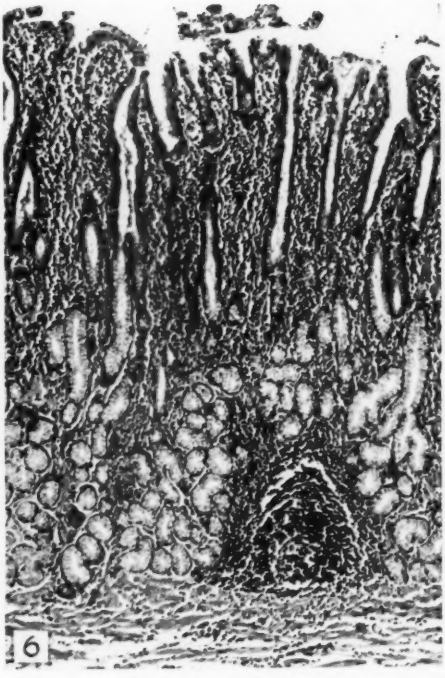
FIG. 5. Normal antral mucosa. Hematoxylin and eosin stain. × 75.

FIG. 6. Antral mucosa showing mild lymphocytic infiltration and a single lymphoid follicle. The glands are intact. Hematoxylin and eosin stain. × 75.

FIG. 7. Antral mucosa showing advanced atrophy, heavy lymphocytic infiltration and follicles. Hematoxylin and eosin stain. × 75.

FIG. 8. Focal area of metaplasia to the intestinal type in an atrophic body mucosa. Hematoxylin and eosin stain. × 75.





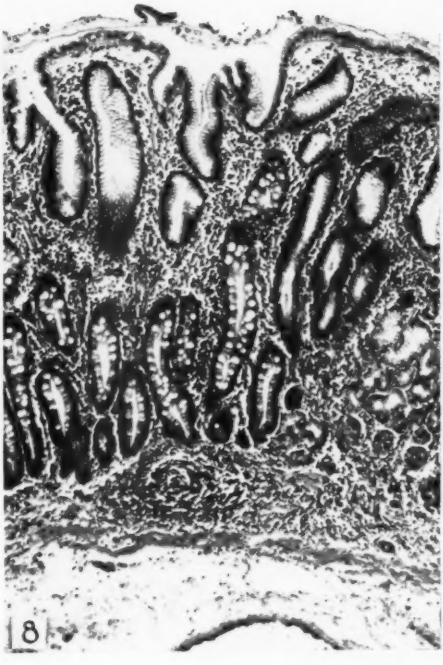


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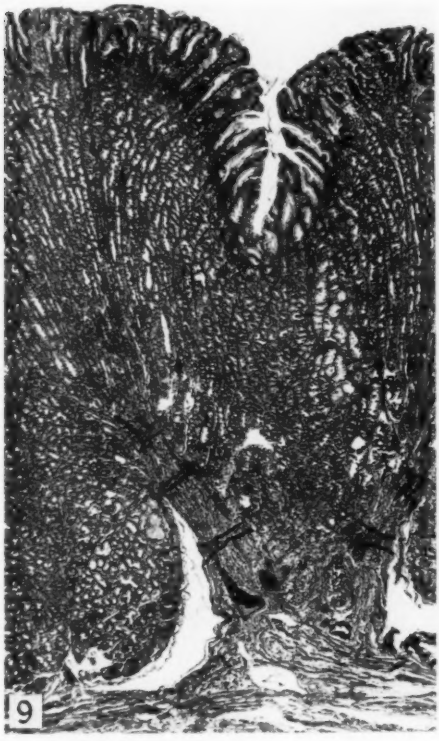
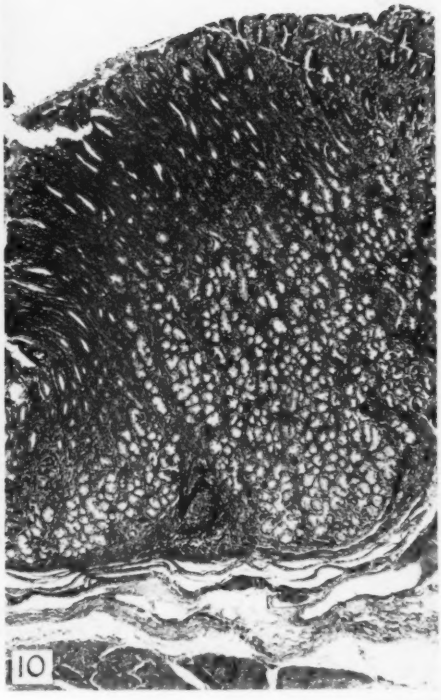
PLATE 9

FIG. 9. Hypertrophic body mucosa. Hematoxylin and eosin stain. × 30.

FIG. 10. Hypertrophic antral mucosa. Hematoxylin and eosin stain. × 30.

FIG. 11. Healed erosion in antral mucosa. Hematoxylin and eosin stain. × 75.





Hebbel Chronic Gastritis

THE LYMPH NODES IN DISSEMINATED LUPUS ERYTHEMATOSUS *

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INTRODUCTION

Enlargement of the superficial and deep lymph nodes, a prominent finding in the majority of reported cases of disseminated lupus erythematosus, has heretofore received only brief mention. In the early literature lymphadenopathy was considered to be pathognomonic, *per se*, of tuberculous infection, and much effort was expended to prove that the "systemic" form of lupus erythematosus was a manifestation of generalized tuberculosis. But in more recent years, largely due to the investigations of Keil (1937) and others, this contention has been found to be untenable.

In 1872, Kaposi reported a group of cases which, he believed, presented a syndrome separate from that of the already well known "discoid type" of lupus erythematosus. He remarked that "in several of the acute cases generalized hard painful swelling of the lymph nodes, especially cervical, axillary and occasionally inguinal" was noted; but in the detailed case reports he mentioned enlarged cervical nodes in only 2 cases, and enlarged cervical and axillary nodes in 1 other case. Only 2 of his 11 cases came to autopsy.

Hardaway (1889), in reporting an example of this disease 17 years after Kaposi's original description, noted "great swelling of the lymphatic glands in the front of the neck." In 1892 he reported another case in a girl of 14 years, and stressed the fact that the "submaxillary glands were swollen somewhat but not excessively."

Although the general impression at the turn of the century was that disseminated lupus erythematosus was caused by the tubercle bacillus, examination of the evidence presented fails to exclude the coincidental occurrence of tuberculosis, a common disease at the time, in an already debilitated patient. Keil (1937) concluded "that the occurrence of tuberculosis in cases of lupus erythematosus is coincidental and unrelated." Sequeira and Balean (1902) found "that the disseminated form of lupus erythematosus is associated with tuberculosis to a much greater degree than the discoid variety," yet the single case which they reported had no evidence of tuberculous infection whatsoever.

Roberts (1911), in reporting a case of disseminated lupus erythematosus, was so impressed by the relationship between the lymphad-

* Received for publication, May 29, 1942.

enopathy and the disease entity that he postulated a theory to explain how this association was affected:

"The relationship [of tuberculosis to lupus erythematosus disseminatus] probably only comes into play when certain organs of the body are attacked by the tubercle bacilli. The researches of Boeck seem to indicate that these special organs are the lymphatic glands. . . . Now, while we cannot admit that lupus erythematosus is a manifestation of tuberculosis or tuberculin poisoning, we are met by the fact that the disease is more frequently associated with adenitis than with any other morbid antecedent, and that this adenitis is very often of a tubercular nature. . . . It is at least conceivable that the cytolytic toxin of lupus erythematosus is derived from the leucocytes in the lymph-glands, and that the formation of the toxin, although not absolutely dependent on the presence of tubercle bacilli, is favored by the presence of the bacillus in the gland. . . . From the first, some depressant influence is at work tending towards lymph stagnation, and it is under this condition that I conceive the origin of the cytolytic poison in, or by, the leucocytes of the lymph-glands."

It is interesting to observe that when the reported cases were reviewed chronologically, the incidence of tuberculous lymphadenitis associated with disseminated lupus erythematosus diminished, although the search, especially microscopically, for tubercle bacilli and tuberculous lesions became more diligent. Scrutiny of the detailed accounts of the early cases of the disease fails to divulge convincing evidence on which to base the concept of these cases as tuberculous. Low and Rutherford (1920) described enlarged mediastinal, para-aortic, mesenteric and cervical nodes in their case, and stated emphatically that evidence of tuberculosis was not found. Ehrmann and Falkenstein reported 6 cases of this disease in 1922; 5 patients had lymphadenopathy, and in only 2 of these was there evidence of tuberculosis; however, even in the 2 positive cases only "some nodes showed tuberculosis, others not." In 1 case, injection of portions of a "tuberculous node" into a guinea pig failed to produce evidence of tuberculosis. Keith and Rowntree, in the same year, reported 4 cases, in all of which there were enlarged lymph nodes. Only 1 case came to necropsy and in this miliary tubercles were found in the spleen. Examination by biopsy of a cervical lymph node in another case revealed tuberculosis. Gennerich (1921), Sibley and Wynn (1923), Gibson (1925) and Clarke and Warnock (1926) were unable to demonstrate evidence of tuberculosis in their cases. Keefer and Felty (1924) described tuberculous lymphadenitis in 2 of their 3 cases, although all had generalized lymphadenopathy. Lyon (1933) failed to produce tuberculous lesions in a guinea pig with portions of enlarged hepatic, cervical and tracheobronchial nodes from 1 of his 2 cases. Baehr, Klemperer and Schifrin (1935) found that "evidences of active tuberculosis were absent in all but two" of their 23 cases. "One had a

single caseous tracheobronchial lymph node. Another had terminal acute miliary tuberculosis." Rose and Pillsbury, writing in 1939, agreed with Keil that "proof of the tuberculous etiology in disseminated lupus erythematosus is lacking, and that the coincidence of active tuberculosis is not necessarily significant." None of our 3 cases (to be described in detail) showed any evidence of tuberculosis in spite of diligent search for specific lesions.

We shall subsequently attempt to show that not only is the absence of tuberculous lymphadenitis the rule, but the morphologic characteristics of the enlarged nodes, independent of blood vessel alterations, are more or less constant and perhaps peculiar to the disease. With

TABLE I

Age, Sex and Color Distribution of 280 Cases of Disseminated Lupus Erythematosus

Age in years	0–10	11–20	21–30	31–40	41–50	51–60	61–70	Age not stated	Total
Females	7	53	73*	39	20	6	4	23	225
Males	2	15	14	7	8	1	0	1*	48
Total	9	68	87	46	28	7	4	24	273†

* One Puerto Rican.

† All patients included were white. Not included are 7 cases: 1 male Hawaiian of Japanese extraction, 25 years of age; 3 Negresses, 24, 35 and 38 years of age, and 3 white patients whose age and sex were not given.

this thought in mind we reviewed most of the reported cases of disseminated lupus erythematosus in an effort to determine the incidence of lymph node enlargement as part of the morbid anatomy, and to gather the gross and microscopic impressions of other observers concerning this alteration.

In our review we paid special attention to lymph node enlargement, either clinically or at necropsy, and to findings which we believe are pertinent to this particular aspect of the disease; namely, splenomegaly, leukocytosis or leukopenia, and morphology of the bone marrow. Our observations, segregated according to age and sex, are tabulated in Table I. We were able to find a total of 277 reported cases, plus the 3 cases to be described. These represented over 250 different cases, after allowing for duplication of cases mentioned by different authors. There were 228 females and 49 males (in 3 cases the sex was not stated). It is significant that 127, or 55.7 per cent, of the females, and 30, or 61.2 per cent of the males, were between the ages of 11 and 30 years, a total of 157, or 62.1 per cent. (In 27 cases the age was not stated.) These figures verify the general impression that the disseminated form of lupus erythematosus is a disease

of adolescents and young adults. There were 214 cases, or 84.6 per cent, in which the patients were under 40 years of age. The youngest case was that of a male infant, 11 months of age, and the oldest a woman of 70 years. Although the disease is one which afflicts the white race predominately, without respect for nationality, and particularly individuals with fair skin, a few cases in Negroes and 1 in an Hawaiian of Japanese extraction were reported.

The Lymph Nodes

General Remarks

The first histologic description of the enlarged lymph nodes in disseminated lupus erythematosus was provided by Short in 1907. He observed that "the sinuses are filled with fibrin and large vacuolated endothelial cells. The lymphoid cells are, to a great extent, replaced by large cells with a considerable amount of protoplasm, the nuclei of which are large and oval, often duplicated, and frequently showing mitotic figures. There are areas of haemorrhage and other areas of complete necrosis."

Low and Rutherford (1920) were impressed by the "separation of the lymphoid elements" in their case "as if from edema"; there was "marked acute hyperemia, with a few minute hemorrhages into the gland substance and much endothelial cell reaction." Denzer and Blumenthal (1937) found changes compatible with acute lymphadenitis in the mesenteric nodes of their case. Madden (1932) remarked that "superficial lymphadenitis is often to be noted, especially in those regions that drain the involved skin. The inflammation in the glands does not go on to suppuration, and recedes when regression of the skin lesions takes place." Ginzler and Fox, as late as 1940, were the first to describe the lymph node alterations in great detail. They remarked that "some lymph nodes showed merely edema, sinus hyperplasia, and an inflammatory reaction manifested by the presence of many histiocytic cells, and a few plasma and polymorphonuclear cells." Many of the nodes in their case "contained numerous areas of necrobiosis, apparently in different stages of development," a finding which was absent from the nodes in our 3 cases. They divided the necrobiotic process into several stages, the earliest consisting of "foci of cellular necrobiosis with virtually no reactive inflammation. These progressed to larger areas of necrosis with complete cell degeneration and disintegration and nuclear pyknosis and karyorrhexis. Nuclear particles were spread throughout the surrounding pulp, which showed marked histiocytic proliferation." Other authors—Short (1907), Keil (1940) and Klemperer, Pollack and Baehr (1941), to

name a few—also mentioned the areas of necrosis, so we are forced to conclude that this change, although not a consistent one, occurs frequently. Ginzler and Fox (1940) felt that "the occurrence of the focal necrotic lesions in the lymph nodes appeared to be as characteristic as the glomerular changes, though undoubtedly of less frequent occurrence." Keil (1937) was of the opinion that the areas of necrosis so frequently observed in the lymph nodes were "formerly used as proof of tuberculous source," which perhaps explain why the association of tuberculosis with disseminated lupus erythematosus was reported so often in the early literature on the subject.

TABLE II

State of Lymph Nodes in 280 Cases of Disseminated Lupus Erythematosus Segregated According to Age and Sex*

Age in years	Enlarged			Not enlarged			Condition not stated		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
0–10	5	2	7	2	0	2	0	0	0
11–20	35	11	46	15	2	17	3	2	5
21–30	37	10	47	17	5	22	19	1	20
31–40	21	5	26	13	1	14	7	1	8
41–50	7	4	11	6	4	10	7	0	7
51–60	3	0	3	1	1	2	2	0	2
61–70	0	0	0	2	0	2	2	0	2
Age not stated	0	0	0	1	0	1	22	1	23*
Total	108	32	140	57	13	70	62	5	67*

* Three cases (age and sex not given) not included in table.

Moderate or marked enlargement of the lymph nodes in disseminated lupus erythematosus occurs too often to be considered a mere coincidental finding. The enlargement may be localized, or generalized, involving the superficial and deep nodes of the entire body (Table III). Of 280 cases, the state of the lymph nodes was mentioned in 210 (Table II). In 140, or 66.7 per cent, there was evidence of lymph node enlargement. These included 108 females and 32 males. We are inclined to view this figure as a conservative estimate of the degree of lymphadenopathy since in 55 of the remaining 140 cases (in which the nodes were not mentioned, or were said to be normal) necropsy was not performed. Tabulation of the enlarged nodes (Table III) shows the cervical chain to be involved most frequently, and the mesenteric, axillary, inguinal and retroperitoneal groups somewhat less so. In 12 per cent lymphadenopathy was generalized. The predominant involvement of the cervical chain of nodes may be more apparent than real; in some patients the cervical enlargement was noted clinically, and many of these patients were still living at the time of reporting,

or died and did not come to necropsy. Nor do we believe that the enlargement of the cervical nodes is related to the facial eruption. This premise would fail to explain the enlarged nodes elsewhere in the body, or the cervical lymphadenopathy in patients without skin lesions (case 2).

Morphologic Description

The enlarged nodes in disseminated lupus erythematosus are usually discrete, and vary in size from 1 cm. in the longest diameter to "large as a fist or even larger." They are soft and succulent, and enclosed

TABLE III

Distribution of Lymph Node Enlargement in 140 Cases of Disseminated Lupus Erythematosus*

Group	Female	Male	Total
Cervical	47	13	60
Mesenteric	23	6	29
Axillary	18	7	25
Inguinal	16	8	24
Tracheobronchial	13	7	20
Generalized	14	3	17
Retroperitoneal	13	3	16
Supraclavicular and infraclavicular	7	3	10
Submaxillary	8	1	9
Pelvic	6	1	7
Epitrochlear	4	3	7
Para-aortic	5	1	6
Mediastinal	1	1	2
Submental	1	1	2
Site not stated	12	5	17

* The totals exceed the number of cases because in most instances more than one group of lymph nodes in the same patient was enlarged.

in a moderately tense capsule. The external surface has a pale gray, shiny appearance. The shape is oval, as a rule. Areas of necrosis of a yellowish or grayish white color may be recognized grossly, and sometimes there are "discrete foci of hemorrhage." Keil (1940) spoke of the "breakdown" of enlarged cervical lymph nodes "with the extrusion of necrotic, often infected, tissue" and likened their appearance to the so-called "scrofulous glands" of tuberculosis. On section, the cut surface of the nodes bulges from the tense capsule which encloses it. The pulp is edematous and almost mushy in consistency. The follicles and trabeculae are obscured in a gray, homogeneous background.

Microscopically, the marked distortion of the lymphoid architecture by edema and engorgement is the most prominent feature (Figs. 1 and 2). Neither primary nor secondary follicles remain. The lymph sinusoids are swollen and distended with lymphocytes, plasma cells,

histiocytes and reticulum cells (Fig. 3). The endothelial cells are also swollen and hyperplastic. Almost the entire vascular bed is engorged, and the capillaries bulge with red cells. The pulp is crowded with lymphocytes, and plasma, mononuclear and reticulum cells. Polymorphonuclear neutrophils are not numerous, and eosinophils are exceedingly rare. The mononuclear cells are swollen and have round, hyperchromatic, granular nuclei. Some contain cytoplastic deposits of nuclear débris. A few reticulum cells may be undergoing mitotic division. Among the numerous plasma cells many have two or even three nuclei (Fig. 4). A constant finding is a large neutrophilic to eosinophilic cell, scattered sporadically throughout the pulp. It is three or four times as large as a lymphocyte, and is not unlike a megakaryocyte. The nucleus is large and multilobulated, and two or more nuclei may be present. The cytoplasm is homogeneous and free of vacuoles (Fig. 5). This cell is not to be confused with the Sternberg cell of Hodgkin's disease, or with the infectious mononucleosis cell described by Gall and Stout (1940) in the lymph nodes of that disease. The capsule, trabeculae and interstices of the node are not thickened, but isolated foci of fibroblastic proliferation may be noted. Some of the smaller arteries and arterioles are surrounded by a cuff of fibrous tissue, an alteration frequently observed around the central arterioles of the spleen (Fig. 6). These vessels may also show the distinctive changes of the disease usually seen in the viscera, particularly the kidneys.

In addition to hematoxylin and eosin, special stains were used on representative sections. No increase in reticulum fibers was found with the aid of Wilder's silver impregnation method, and Weigert's differential stain failed to disclose the presence of fibrin. Neither Masson's trichrome stain for connective tissue nor Mallory's phloxine and methylene blue stain revealed any features not noted in the hematoxylin and eosin preparation.

Related Data

Spiethoff, in 1912, was the first to investigate the peripheral blood of patients afflicted with disseminated lupus erythematosus. He reported 2 cases, 27 and 20 years of age, respectively. In 1, the white cell count ranged from 3,400 to 4,600 cells per cmm., never rising above 5,300. In the other, the initial count was 6,100 white blood cells per cmm., rising to 6,950 soon after. It then fell to 5,100, rose to 5,600 and reached 9,450 just before death. In 1915, Spiethoff commented briefly on the absence of leukocytosis and the tendency to leukopenia in this disease. Subsequently this feature was noted by

others, more and more frequently, and undoubtedly is part of the syndrome of disseminated lupus erythematosus. Of 142 cases in which white blood counts were performed, 90 had leukopenia (less than 5,500 white blood cells per cmm.) at some time during the course of the illness. In many there was a distinct tendency towards leukocytosis during the terminal phase of the disease, contradicting Madden's statement that "the leucocyte count was lower just before death than at any other time." The white blood count usually hovers around 4,000 cells per cmm., occasionally falling below 3,000, or rising above 5,000. In Madden's sixth case, that of a girl of 20 years, the initial count of 4,000 white blood cells per cmm. rapidly fell to 1,200 and finally descended to 350. Although counts as low as this are not common, it tends to emphasize the degree of leukopenia which might be expected. The differential count usually has a normal distribution, and eosinophilia is not a feature. Friedberg and Gross (1936), and others, commented upon the association of thrombopenia with some cases of disseminated lupus erythematosus. These usually displayed moderately severe leukopenia, but the latter finding was by no means limited to the cases with thrombopenia.

Study of the bone marrow in disseminated lupus erythematosus has, perhaps, not received sufficient attention. The few comments on the state of the marrow, culled from the literature, are conflicting and inadequate. Certainly thrombopenia is not constantly associated with aplasia of the marrow. In Friedberg's second case (E. K.), which displayed marked thrombopenia (80,000), "the bone marrow from a vertebra showed activity, with many myeloid cells of all types." Templeton (1934) remarked that aplasia was noted in the marrow of his first case, in which the platelet count fell to 70,000. Baehr, Klemperer and Schifrin (1935) thought "perhaps due to toxic damage to bone marrow, or to vascular lesions in the marrow, the blood picture usually reveals evidences of a depression in bone marrow function, leukopenia, and a moderate anemia." In 1941, Klemperer, Pollack and Baehr reported 20 selected cases of disseminated lupus erythematosus and commented to the effect that "the bone marrow showed no changes of significance." Denzer and Blumenthal (1937) found the "bone marrow very cellular and the ratio of bone marrow cells normal" in their case. Ginzler and Fox (1940) said the red marrow in their case "showed definite hypoplasia of the blood-forming elements, though all were present in normal proportions. The marrow revealed no vascular alterations or areas of necrosis." A sternal biopsy in case no. 24201 of the Cabot series (1938) showed hyperplasia of the marrow. Banks (1941) believed that "there is often depression

of the bone marrow" in cases of disseminated lupus erythematosus, although in his case the marrow was not mentioned. The marrow (sternum) was examined in only 1 of our 3 cases (case 2) and failed to show any dyscrasia to account for the almost constant leukopenia.

Splenomegaly is not a feature of disseminated lupus erythematosus and "is best remembered in a negative way." In the cases reviewed in this paper, the spleen was mentioned specifically in 220, and in only 81 (36.8 per cent) of these was it enlarged. In none was the enlargement massive. Only 3 of the 20 cases of Klemperer, Pollack and Baehr had spleens which weighed over 300 gm. The average weight was 260 gm. In our 3 cases the spleens weighed 220, 240 and 218 gm. Except for frequent evidence of perisplenitis and focal infarction, there were no characteristic changes reported in the literature. A few authors noted miliary tubercles as part of a generalized state of miliary tuberculosis. Microscopically, two features were mentioned frequently, although not always in combination. These were areas of focal necrosis, similar to those described in the lymph nodes, and "a peculiar periarterial fibrosis limited to the central and penicilliary arteries." Klemperer, Pollack and Baehr (1941) gave prominence to the latter feature, having noted it in 19 of their 20 cases. "In cross sections of these vessels (central and penicilliary arteries) the fibrosis assumes a pattern of concentric rings of stout collagen fibers, with few intercalated fibroblasts." The necrotic areas are formed of poorly circumscribed zones of cellular débris. The nuclei of the cells which remain are pyknotic and karyorrhectic. At the periphery of such zones, the necrotic elements gradually blend with the surrounding nonnecrotic pulp, without forming any sharp line of demarcation.

Reports of Cases

We wish to record 3 cases of disseminated lupus erythematosus, all of which had more or less generalized lymphadenopathy without evidence of tuberculosis. One case (L. P.) is of especial interest because of the absence of skin lesions (lupus sine lupus). It was the marked lymph node enlargement in this particular case which focused our attention upon the rôle of the lymph nodes in the disseminated form of lupus erythematosus.

Case 1

H. P. (N.B.G.H. 93782*), a white, native-born female, 24 years of age, enjoyed good health until 1937, when she developed a "crusting lesion" of the skin of the face. At that time she was suffering from "an infected tooth." A dermatologist con-

* We are indebted to Dr. John C. White of the New Britain General Hospital for permission to report this case.

sidered the skin condition to be "acute lupus erythematosus." She had had measles, mumps and whooping cough during childhood. No history of close contact with tuberculosis, or of rheumatic fever was elicited. The family history was noncontributory. Intramuscular injections of 3 per cent bismuth sodium tartrate were administered weekly until March, 1938, when the skin lesion disappeared. Three months later, a similar skin eruption appeared beneath the left eyelid and slowly spread to adjacent areas. Eighteen additional injections of the bismuth preparation were given without beneficial effect. In June, 1939, complete physical examination revealed a well developed and well nourished female with soft white skin. There was an erythematous crusting lesion over the bridge of the nose extending to both cheeks. The eyes and eyegrounds were normal. No abnormalities were noted in the heart or lungs and this was confirmed by x-ray examination. Blood pressure was 110/78 and pulse 85 per minute. The liver and spleen were not palpable. Rectal examination was negative. The tendon reflexes were equal and "very active." A small, nontender lymph node was felt in the right axilla. Roentgenograms of the teeth failed to show evidence of infection.

Laboratory tests at this time showed the hemoglobin to be 70 per cent (Newcomber), a "normal" urine and a negative flocculation test (Hinton). A tuberculin patch test was "faintly positive." In the spring of 1939, gold sodium thiosulfate and vitamins B and D were given; the patient was instructed to avoid exposure to sunlight. In addition, increasing doses of chaulmoogra oil were given intramuscularly, beginning with 1 cc., but were discontinued after the fourth injection because of a "painful, swollen, hot, indurated area which formed in the right buttock," associated with edema of the right lower extremity. Ten days after the last injection of chaulmoogra oil the patient developed pitting edema of both lower extremities, tenderness of the skin of the feet and ankles and a generalized maculopapular, pruritic eruption. Sulfanilamide, 20 grains, was given daily, for 6 days, without improvement. At this time enlarged, tender lymph nodes appeared in the epitrochlear, axillary and inguinal regions. The patient was admitted to the New Britain General Hospital on October 30, 1939, because of increasing weakness and malaise, associated with dizziness, swelling and tenderness of the right wrist and severe pain in the extremities. Her throat was "sore," and she had "bleeding gums." The temperature was 99° F.; pulse, 86 per minute; respiration, 20 per minute. There was a florid, crusted erythema of the forehead, nose and cheeks and a crusting, weeping eruption of the hands. The generalized rash had disappeared. The mucous membrane of the mouth was pale and the gums bled easily. Diffuse, sonorous râles were heard throughout both lung fields, without impairment of resonance or breath sounds. The heart was "normal." The spleen was not palpated, nor were there any swollen or tender lymph nodes at this time. The peripheral edema had disappeared, but marked tenderness was noted over the left heel, right wrist and right shoulder.

Laboratory Data. Hemoglobin, 66 per cent (10.0 gm.); red blood cells, 3 million per cmm.; white blood cells, 9,800 per cmm., with 65 per cent polymorphonuclear neutrophils, 31 per cent lymphocytes, 2 per cent eosinophils, 2 per cent monocytes. Sedimentation rate (Westergren), 120 mm. in 1 hour. Urine: specific gravity, 1.018; albumin, trace; red blood cells, few; occasional granular cast per low-power field. Transfusions of whole blood were given, and 10 days after admission to the hospital the hemoglobin was 69 per cent (10.5 gm.); red blood cells numbered 3 million per cmm.; white blood cells, 6,900 per cmm., with 63 per cent polymorphonuclear neutrophils, 34 per cent lymphocytes and 3 per cent eosinophils. Two days later the hemoglobin was 72 per cent (11.0 gm.); red blood cells, 4 million per cmm.; white blood cells, 6,500 per cmm., with 79 per cent polymorphonuclear neutrophils, 20 per cent lymphocytes and 1 per cent eosinophils. Two blood cultures were sterile. The blood nonprotein nitrogen was 123 mg. per cent and the creatinine, 2.5 mg. per cent.

Course. The temperature fluctuated between 99° and 103° F., rising to 105° on one occasion. Supportive treatment in the form of vitamin concentrates, diet and small repeated blood transfusions was given. Roentgenograms of the chest, 12 days following admission, revealed "bronchopneumonia" at the base of the left lung. Appetite diminished and somnolence increased, accompanied by involuntary twitchings. The patient died on the 17th hospital day.

Necropsy

The necropsy was performed 2 hours after death. Only the pertinent findings are recorded.

External Examination. Over the skin of the face there was a dry, scaly, blanched area with indefinite borders. It covered the bridge of the nose and both cheeks in butterfly fashion. Similar patches were found in the temporal regions, bilaterally, stopping abruptly at the hairline. There was no fissuring or loss of surface epithelium. A few similar patches of small size were found in the skin of the forehead, chest, and over the extensor surfaces of the arms, forearms and fingers. The skin of the entire back was peppered with slightly raised, punctate, maculopapules, most numerous over the bony thorax. The superficial lymph nodes were not palpable.

Internal Examination. The *heart* weighed 310 gm. There was no evidence of hypertrophy, thrombi, or valvular defects. The right and left *lungs* weighed 630 and 510 gm., respectively. The visceral pleura of the right lung was covered by strands of gray fibrin, especially over an accessory fourth lobe, situated between the right upper and middle lobes in the axillary line. It was solid and noncrepitant. The cut surface was engorged, gray-red and granular. Palpation disclosed numerous small, nodular areas, deep within the lung substance, which on section were slightly raised, bluish red, granular zones. Yellow pus exuded from the smaller bronchi and bronchioles in the centers of the granular areas mentioned above. These granular lesions were most numerous throughout the lower lobes of both lungs. There was no scarring or evidence of tuberculosis. The *liver* was swollen and weighed 1820 gm. It measured 30 by 21 by 7 cm. The external surface was mottled yellow-red; the cut surface was marked by scattered, small gray areas of necrosis, situated in a yellowish red background. The *spleen* was large and soft. It weighed 220 gm. and measured 14.5 by 8 by 3 cm. The capsule was smooth and tense; the cut surface bulged away from the capsule and revealed numerous, well defined, large gray malpighian corpuscles. At one pole there was a well circumscribed, necrotic gray zone, 1 cm. in diameter, as well as other smaller, similar zones. The pulp was deep red and soft. The *right kidney* weighed 220 gm. and measured 13 by 6.3 by 3 cm. The *left*

kidney weighed 230 gm. and measured 12.5 by 6.5 by 4 cm. A soft, wedge-shaped, gray-yellow zone was found in the cortex of the left kidney. The *lymph nodes* surrounding the aorta and those situated in the mesentery of the small intestine were all discretely enlarged, tense and succulent. The external surface was reddish pink, smooth and moist. On section the cut surface was homogeneously gray and moist. The follicle markings were obliterated, and there was no evidence of necrosis.

Microscopic Examination. No vascular changes or evidence of infiltration were noted in the *heart*. The bronchioles of the *lungs* were distended with an exudate consisting principally of polymorphonuclear cells, mixed with desquamated bronchiolar epithelium. The exudate extended from the bronchioles to involve the surrounding alveoli, and was also noted on the pleural surface. The malpighian corpuscles of the *spleen* showed extensive changes. The central arterioles were reduplicated and their walls were irregularly thickened. The outstanding finding, however, was thick collars of homogeneous, pink, acellular, periarterial connective tissue. The central arterioles were situated within these dense fibrous zones which occupied the major portion of the malpighian bodies. Some arterioles had deep red mural deposits of fibrinoid material, but the lumina were usually widely patent. A single large zone of necrosis, involving both the red and white pulp, was noted; at its periphery there was a wide band of hemorrhage and congestion. A small *accessory spleen* showed similar changes. There was no obvious scarring of the *renal cortex,* but the glomerular changes were very striking. Many endothelial cells lining Bowman's capsule were swollen, and there was rare capsular crescent formation. Bowman's space was filled with pink granular material in some places. Most of the glomerular tufts were free, although occasionally definite adhesions were present between them and the capsule. The prominent changes, however, were in the glomerular tufts, which were large and swollen. The swelling was not due to cellular proliferation, but rather to the deposition of deep pink-staining, collagenous tissue. Under subdued light, the connective tissue thickening of the basement membrane conformed to the typical "wire loop" design described in disseminated lupus erythematosus. Only rarely was a bright red focus of fibrinoid material seen within the glomerular tuft. The tubular epithelium was universally swollen and granular, and the lumina were filled with pink granular débris. No alteration of the afferent arterioles or medium-sized arteries was noted. A section of *skin* from a scaly lesion on the chest wall had a thin epidermis surmounted by a thin layer of keratin. Beneath the basal layer there

were scattered large mononuclear cells containing brown pigment, resembling hemosiderin. In addition, a small focus of lymphocytes and plasma cells was noted in the dermis. There was no sign of acute inflammation. All the *lymph nodes* examined were similar, but the structure of one node in the peripancreatic group was so completely disorganized that it was not possible to distinguish lymph follicles. This was due to the widespread edema which separated adjacent cells, associated with marked engorgement. The outstanding feature, however, was the diffuse increase in the number of reticulum cells. Areas of necrosis were not seen.

Anatomic Diagnosis. Scaly erythematous lesions of skin of face, anterior chest wall and arms; acute necrotizing arteriolitis of spleen and kidneys; hyperplasia of abdominal lymph nodes; focal pneumonia. (The anatomic findings were consistent with the clinical diagnosis of disseminated lupus erythematosus.)

Case 2

L. P. (N.B.G.H. 104075*), a white, Swedish female, 35 years old, was admitted to the New Britain General Hospital on April 22, 1941, complaining of "swelling of the glands of the neck." She stated that for 1½ years prior to admission she had had painful, symmetrical swelling of the middle joints of the fingers of both hands. During the past year she suffered from "arthritis of the neck, shoulders, spine and hips." Three months before admission she received "a course of gold injections" for the arthritic symptoms, without relief. She had the usual "childhood diseases." In 1935, an operation was performed for some gynecologic disorder, the nature of which was not known. The appendix was removed at that time. In 1937, she was treated for "an ulcer of the rectum." Her father died of "carcinoma of the pancreas"; her mother and seven siblings were living and well. Her husband and two children were also living and well.

Upon admission to the hospital the skin of the entire body was "clear." There was bilateral clubbing of the fingers and slight hypertrophy of the middle joints. Symmetrical nodular swelling of the preauricular, postauricular and superficial and deep cervical lymph nodes was noted. Roentgenograms of the chest showed "enlargement of the left auricle of the heart, and pulmonary congestion."

Laboratory Data. Hemoglobin, 57 per cent (8.7 gm.); red blood cells, 3.0 million per cmm.; white blood cells, 4,300 per cmm., with 68 per cent polymorphonuclear neutrophils, 27 per cent lymphocytes and 5 per cent monocytes. Sedimentation rate (Westergren method), 39 mm. in 1 hour. Urine: specific gravity, 1.006; albumin, +++; red blood cells, 175 to 200 per high-power field; 25 to 35 leukocytes per high-power field.

Course. Three days after admission a cervical lymph node was removed for diagnosis; it showed "lymphoid hyperplasia, nonspecific." Temperature ranged around 100° F., rising to 102.4° F. on one occasion. The pulse fluctuated between 70 and 90 per minute, respirations were 20 per minute and blood pressure was 162/100. The patient was discharged unimproved 4 days later.

Final Admission. The patient was readmitted to the New Britain General Hospital on May 20, 1941, complaining of a "choking sensation" beneath the lower

* We are indebted to Dr. Roger T. Scully of the New Britain General Hospital for permission to report this case.

sternum, associated with difficulty in breathing. These symptoms occurred at intervals of 3 to 4 days and would last for about 24 hours. There was intermittent nonproductive coughing, and on one occasion "rather profuse" hemoptysis. At this time the temperature was 99° F., pulse 90 per minute and respiration 20 per minute. Blood pressure was 120/90. A faint systolic, apical murmur was heard "occasionally." Multiple "petechiae" were found on the hard palate, and skin of the trunk. Bilateral costovertebral-angle tenderness was noted, and Murphy's sign was positive.

Laboratory Data. Hemoglobin, 40 per cent (6.1 gm.); red blood cells, 2.3 million per cmm.; white blood cells, 3,300 per cmm., with 63 per cent polymorphonuclear neutrophils, 32 per cent lymphocytes, 2 per cent eosinophils and 3 per cent monocytes. Sedimentation rate (Westergren), 70 mm. per hour. Urine: specific gravity, 1.004; albumin, ++; 125 to 150 red blood cells per high-power field; 20 to 25 leukocytes per high-power field; 6 to 7 granular casts per low-power field. The blood nonprotein nitrogen was 50 mg. per cent and the creatinine, 1.8 mg. per cent. Repeated blood cultures were sterile after 15 days.

The temperature ranged from 99° to 101° F. for 10 days, rising to 102° or 103° F. for a period of 1 week. It then fell to 99° F. for 5 days, only to rise precipitously to 103° F., reaching 104.6° F. on one occasion. From this time until death it continued at about 102° F. with minor fluctuations. The pulse rate more or less paralleled the temperature. The patient's condition became progressively worse. Numerous pyogenic cutaneous lesions appeared, particularly around the eyelids. At no time was any skin rash noted. She died on July 25, 1941, approximately 17 months after the onset of symptoms.

Necropsy

The necropsy was performed 9½ hours after death.

External Examination. The body was that of a well developed, but poorly nourished, white female of 35 years. The hair was reddish blonde; the skin was pale and clear; a few ecchymotic areas were found over the anterior chest wall and upper abdomen, but there were no petechiae. The posterior auricular and superficial cervical lymph nodes were moderately enlarged. The fingers were fusiform in shape, and clubbed.

Internal Examination. The right and left pleural cavities contained 200 and 100 cc. of clear, straw-colored fluid, respectively. The visceral pleura over the lower lobe of the *right lung* was partly covered by flakes of yellow fibrin. The right lung weighed 770 gm. The lower lobe was firm; the middle and upper lobes were crepitant and flabby. On section, the cut surface of the right lower lobe was reddish pink, and contained many poorly circumscribed, small gray-yellow areas. On pressure, thick yellow pus exuded from these areas. The *left lung* weighed 420 gm., and its external surface was smooth and glistening. Both the upper and lower lobes were crepitant. The *heart* weighed 270 gm. Except for slight thickening along the line of closure of the mitral valve, no valvular deformities were noted. The *peritoneal cavity* contained about 300 cc. of clear greenish fluid. The *appendix* was

absent. The *liver* weighed 1530 gm. and measured 23 by 19 by 7 cm. The *spleen* weighed 240 gm. and measured 14 by 9 by 6 cm. It was flabby in consistency; the capsule was bluish gray and wrinkled. On section, the cut surface was blue-red and studded with innumerable tiny gray flecks. The pulp could be scraped away easily. The *kidneys* were similar to each other. The right weighed 190 gm. and measured 13 by 6 by 3.5 cm.; the left weighed 220 gm. and measured 13 by 7 by 4 cm. The smooth, glistening capsules were stripped with ease, revealing a shiny, brick-red, lobulated cortical surface. Scattered over the cortical surfaces there were innumerable tiny hemorrhagic dots and streaks. On section, the cortex was 5 mm. in average thickness; the cut surface of both cortex and medulla was marked by hemorrhagic foci, similar to those on the external surface. The pelves and ureteropelvic junctions were not dilated. The mucosa of the slightly dilated *urinary bladder* was marked by several large, poorly circumscribed, hemorrhagic areas. The *pelvic organs* were all present and unaltered. The tracheobronchial, inguinal, pelvic and iliac groups of *lymph nodes* were enlarged, soft and succulent; the largest measured 2.5 cm. in longest diameter. The external surface was smooth, moist and reddish gray. On section, the cut surface had an edematous injected appearance. The lymph nodes in the region of the common bile duct and second portion of the duodenum, were also enlarged, as were those in the region of the stomach.

Microscopic Examination. The *mitral valve* was moderately thickened by loose cellular connective tissue in which there were small foci of lymphocytes. Near the base of the cusp the nuclei of the fibroblasts were arranged in palisade formation. Along the free border, opposite the area of palisading, there were a few "Anitschkow myocytes." Sections from the lower lobe of the *right lung* showed the alveoli to be completely filled with exudate composed of polymorphonuclear neutrophils, lymphocytes, mononuclear cells, fibrin and precipitated fluid. The lumina of the smaller bronchi and bronchioles were filled with a similar exudate. Isolated foci of Gram-positive cocci, arranged in chains, were found throughout the exudate. The malpighian corpuscles of the *spleen* appeared to be reduced in size and number. The walls of many of the central arterioles were thickened, without luminal narrowing, by homogeneous eosinophilic material. With Masson's trichrome method the inner portion of the vessel wall stained reddish purple and the outer portion green. Around some of the arterioles there was a wide cuff of fibrous connective tissue. The red pulp was engorged and diffusely infiltrated by polymorphonuclear neutrophils, lymphocytes, plasma cells and mononuclear cells. Several large cells

with single, round, or multilobulated hyperchromatic nuclei were also present. There were no areas of necrosis. Sections from both *kidneys* showed the glomeruli to be markedly altered by fusion of the tufts, both to each other and to Bowman's capsule. In a majority of the glomeruli, one or more, often two or three, tufts were converted into an eosinophilic granular necrotic mass in which all cellular detail was obscured. Portions of the conglomerate mass had a bluish hue. In some glomeruli, tufts at opposite poles showed these alterations and the intervening tufts were relatively unchanged. The basement membrane of many tufts was thickened so that the outlines were clearly visible, in sharp contrast to the surrounding, more or less indiscriminate mass. This accentuation of the basement membranes emphasized the "wire loop" picture described by Baehr, Klemperer and Schifrin (1935). The capillaries of the uninvolved tufts were engorged, and surrounded by lymphocytes and polymorphonuclear neutrophils. Bowman's capsule, for the most part, was not thickened. However, in some places so much connective tissue thickening had occurred that the capsular space was completely obliterated and the glomeruli compressed. The afferent arterioles and juxtaglomerular bodies appeared to be unaltered. The interlobular and arcuate arteries were apparently spared. The intertubular stroma throughout both cortex and medulla was diffusely infiltrated by lymphocytes, plasma cells and a few polymorphonuclear neutrophils. The tubular cells were not altered although many tubules contained precipitated albuminous material. With Masson's trichrome method the eosinophilic glomerular masses stained red, suggesting necrosis. The thickened basement membrane of the glomerular tufts stained green, accentuating their "wire loop" appearance. The latter was also demonstrated by stains for elastic tissue and by van Gieson's mixture. In sections of the enlarged *lymph nodes* the architecture was distorted by marked engorgement of the blood vessels and edema of the lymph sinuses. Neither primary nor secondary lymph follicles were present. In addition to the lymphocytes usually seen in the pulp, there were innumerable plasma and large mononuclear cells. Some of the plasma cells had two nuclei. The nuclei of many of the large mononuclear cells were hyperchromatic, multilobed, and a few were undergoing mitosis; the cytoplasm was basophilic. There were also many reticulum cells. The wall of a small arteriole, in a section of inguinal lymph node, was thickened by eosinophilic hyaline material, like that described in the kidneys. A few arterioles in iliac nodes contained reddish blue granular plugs; their walls were thickened by connective tissue, with slight perivascular fibrosis. There was no apparent diminution

in the cellularity of the *sternal bone marrow* to account for the leukopenia noted clinically.

Anatomic Diagnoses. Primary. Acute necrotizing arteriolitis of kidneys and spleen; generalized enlargement of lymph nodes; fibrinous pleurisy and lobular pneumonia; acute splenic tumor; chronic cystitis; hydrothorax, bilateral; hydropericardium; ascites. *Subsidiary.* Fibrous thickening of mitral valve; scar of abdominal skin; absence of appendix. (The anatomic findings were consistent with the clinical diagnosis of disseminated lupus erythematosus sine lupus.)

Case 3

L. M. (N.H.H. A76000*), white, American-born female, 16 years old, first developed "a pink wrinkled spot on the nose at the age of 7 years." When she was 14 years old the lesion, which had remained stationary until then, turned brown and became scaly. In February, 1937, at the age of 16 years, the posterior cervical and occipital lymph nodes became swollen,. In May of that year, a barber noted that the patient had "eczema of the scalp and falling hair." One month later, red, swollen, itching patches developed on the cheeks and spread rapidly to involve the entire face except for the circumoral region. The eruption became scaly, soon extended to the shoulders and somewhat later to the upper thorax and arms. During this time swelling and pain in the "ankles, knees, hands and back" became manifest, and in August, 1937, easy fatigability, shortness of breath, nocturnal fever, anorexia, and red spots on the fingers and palms were noted.

The past history was noncontributory. Her mother had an area of pigmentation over the jaw, bilaterally, which had developed during pregnancy.

The patient was admitted to the New Haven Hospital for the first time on August 31, 1937. She had fever (103° F.), enlarged heart with tachycardia (124), gallop rhythm and incomplete atrioventricular block. There was generalized glandular enlargement. The spleen was not palpable. On September 7th the red blood cells numbered 2.87 million per cmm. with 62 per cent hemoglobin; white blood cells, 6,300 per cmm.; polymorphonuclears, 83 per cent; lymphocytes, 16 per cent; basophils, 1 per cent. After three blood transfusions the patient's serum agglutinated the cells of all prospective donors. Treatment consisted of liver, iron and, later, nicotinic acid. Two weeks after admission the skin lesion appeared to be receding and showed evidence of pigmentation. Three months later the eruption blossomed again, accompanied by hematuria, albuminuria, nausea, vomiting, ascites and pericardial effusion. The blood nonprotein nitrogen rose to 100 mg. per cent, but rapidly fell to 36 mg. per cent in 2 weeks' time. She was discharged from the hospital in April, 1938.

At home her condition seemed to improve, and she gained 10 lbs. in weight. During the latter part of May, 1938, however, she suddenly experienced severe stabbing precordial pain, precipitated by deep respiration. There was associated fever and weight loss, as well as recurrence of the skin lesion. She was readmitted to the hospital on January 14, 1938. At this time her heart measured 21 cm. in transverse diameter, as determined by roentgenograms, and the lower lobe of the left lung was "atelectatic." During her hospital stay the cardiac dimensions decreased slowly, and the skin lesion faded. She was discharged on August 12, 1938.

The patient was then ambulatory for approximately 6 months, during which time she was troubled occasionally by arthralgia of the knees and fingers. The precordial

* We wish to thank Dr. Francis G. Blake of the New Haven Hospital for permission to report this case.

pain and dyspnea gradually returned, as did the "rash of the face and hands." The third admission to the hospital was on March 11, 1939. The cardiac findings were similar to those of the previous admission, plus a systolic murmur in the mitral area. Following discharge on April 12, 1939, she suffered from "mumps and poison ivy."

She was readmitted to the hospital on December 29, 1939, because of swelling of the ankles of 1 month's duration. Hypertension (180/110) was noted for the first time; the cardiac findings were unchanged. The blood nonprotein nitrogen was 51 mg. per cent; serum protein, 4.26 per cent, with reversed albumin-globulin ratio; urinary albumin, +++. On a salt-free, high protein diet the edema disappeared, and she was discharged on February 2, 1940. A few days later the edema reappeared and persisted. It was accompanied by dyspnea, orthopnea, anorexia, nausea, vomiting, epigastric discomfort, cough, restlessness, and thirst.

Final admission to the hospital was on February 23, 1940. Temperature was 100.2° F., pulse 100 per minute, respirations 24 per minute and blood pressure 144/112. On examination, the patient, who was now 19 years of age, was found to be chronically ill, with dry scaling, brown pigmentation of the face, distributed in butterfly pattern over the bridge of the nose. Elsewhere the skin was clear but dry. Signs of focal pneumonia were found. There was gallop rhythm, and a systolic murmur at the apex of the heart. The lower border of the liver extended 5 cm. below the right costal margin. The abdomen was distended and a fluid wave was elicited. There was pitting edema of the lower extremities and presacral region. The blood nonprotein nitrogen was 102 mg. per cent; serum protein, 5.8 per cent, with reversed albumin-globulin ratio and marked albuminuria. The sedimentation rate was not elevated. Digitalis and supportive therapy were given without relief. She became anuric on the fourth hospital day, and died 3 days later.

Necropsy

The necropsy was performed 1 hour and 40 minutes after death.

External Examination. The *skin* of the face was dry and scaly, with small, pale brown areas distributed in mottled fashion. The discoloration was absent around the alae nasae, over the chin, eyelids and base of the nose. The dryness and discoloration were also noted over the scalp, but stopped abruptly just beneath the mandible, did not extend over the neck nor over other parts of the body. The skin of the thorax and upper extremities was dry and loose; over the lower extremities it was tightly stretched, and there was moderate subcutaneous edema. There were no palpable lymph nodes in the cervical, supraclavicular, epitrochlear or inguinal regions; several small, firm, easily movable nodes were palpated in each axilla.

Internal Examination. The *pericardial cavity* contained 450 cc. of clear yellow fluid. There were numerous dense adhesions around the spleen and over the surface of the liver. The *heart,* together with the pericardium and bases of the great vessels, weighed 540 gm. The epicardium was thick and opaque. The pericardial space was completely obliterated by fibrous adhesions. The right atrium and ventricle were moderately dilated. The mitral valve was thickened and rolled along the free margin. The anterior cusp was quite short and bound

down to the ventricular endocardium by thin adhesions. The chordae tendineae of this cusp were thick and fused, as were the papillary muscles. The *right* and *left* lungs weighed 235 and 188 gm., respectively. Thick fibrous pleural adhesions were found. Each pleural cavity contained about 140 cc. of clear yellow fluid. Externally the lungs were pale pink, mottled by gray lines and flecks. The lungs were soft to palpation, except the left lower lobe, which was moderately crepitant. On the lateral aspect of the right upper lobe there was a brown-red, wedge-shaped area, 2 cm. in diameter. The *spleen* weighed 218 gm. and measured 12 by 7.5 by 4 cm. The external surface was dull purple, and the capsule was roughened by tags of fibrous tissue. The malpighian corpuscles stood out in the cut surface as small gray nodules, surrounded by soft pulp which could be scraped away easily. The *liver* weighed 1605 gm. and measured 25 by 19 by 8 cm. Each *kidney* weighed 180 gm. The right measured 11 by 6.5 by 4, and the left 10 by 7.5 by 4 cm. The capsules were stripped easily, revealing a mottled pink cortical surface, peppered by protruding, white, round areas, less than 1 mm. in diameter. Among these were small red dots, suggesting petechiae. The cut surface was similar to the external surface. The calyces, pelves and ureters were not dilated. There were several small, firm, easily movable nodes in the abdominal cavity and axillae; all showed evidence of pigmentation and hyperplasia. The *calvarium*, which transmitted light, contained considerable red marrow.

Microscopic Examination. A section of *heart* showed the small arteries of the myocardium to be markedly thickened so that the lumen was reduced to a narrow slit. However, the musculature was well preserved. Two small necrotic foci were present in which the muscle fibers were replaced by faint, pink-staining, granular material, surrounded by a few scattered lymphocytes. The smaller arterial branches in the *lung* sections revealed a striking degree of mural thickening, with marked reduction of the lumina. Sections from the right upper lobe showed foci of pneumonia. The malpighian corpuscles of the *spleen* were large. Their central arterioles had very thick walls of dense connective tissue, and many vessels were surrounded by increased fibrous tissue, in some cases involving most of the malpighian corpuscles. There were no necrotic foci. The small arteries of the *pancreas* also showed marked eccentric intimal thickening due to dense connective tissue. The *liver* was not remarkable. The small arteries in the *periadrenal* fat were thickened.

Marked changes were noted in the arteries and arterioles of the *kidneys*. The larger vessels showed advanced eccentric intimal thickening by loose connective tissue. The smaller arteries, however, were

altered by a necrotic process in varying stages of development. In some places the wall consisted almost entirely of bright, pink-staining, fibrinlike material, which was infiltrated by polymorphonuclear cells. Some vessels were rimmed by increased connective tissue and lymphocytes. Practically all glomeruli were surrounded by a thick ring of dense connective tissue, representing all stages of fibrotic change, including obliteration of the glomerulus; some were hyalinized. A few showed proliferation of Bowman's capsule, and many were unusually small. Isolated glomeruli were quite large, and their capillaries were engorged. The convoluted tubules varied strikingly, a cluster of them being widely dilated, and an adjacent group compressed by scar tissue. Many contained a small amount of pink granular material. The epithelium of the *urinary bladder* was not unusual. The muscle and subserosa contained small arteries which showed striking arteritis and periarteritis. One artery had a pinpoint lumen surrounded by a thick wall of granular necrotic material; the latter stained bright pink and had the appearance of fibrin. There were a few polymorphonuclear neutrophils in the wall, and perivascular clusters of lymphocytes. The wall of another vessel was composed of gray, necrotic, amorphous material.

The sinusoids of an abdominal *lymph node* were distended and relatively free of cells. Here and there eosinophils and large phagocytic cells were noted. The medullary cords and margins of the sinusoids were dotted by large numbers of mononuclear cells which were filled with brilliant brown pigment. The capillaries throughout the section were engorged. The *tongue* was the site of an acute inflammatory process; there were areas of necrosis and intense polymorphonuclear cellular infiltration in the superficial musculature. A section from the *rectus abdominus muscle* presented a small artery surrounded by densely packed lymphocytes. Some of the muscle fibers, seen only on cross section, stained bright pink; stippling, clearly seen in the less deeply stained fibers, was absent. The *skin* from the region of the left ear showed areas of brown pigmentation. The epidermis was thin, consisting of a basal layer, a narrow strip of stratum spinosum, and a single row of granular cells, surmounted by a rather thick layer of keratin. There was marked parakeratosis; almost all of the spinous layer consisted of thin, poorly demarcated, elongated cells with pink cytoplasm. In the dermis, close to the basal layer, there was a row of small mononuclear cells containing light brown granular pigment. Several small foci were seen in the subepithelial layers of the dermis in which the connective tissue stained deep pink and appeared hyalinized. There was moderate lymphocytic concen-

tration around some of the hair follicles and small arteries. Small hemorrhages were seen in the superficial portion of the mucosa of the *colon*. Many small arteries of the muscularis and subserosa had thickened walls and small lumina. One such vessel contained a large mass of connective tissue, probably an organized thrombus. The wall of this vessel was infiltrated by lymphocytes and plasma cells.

Anatomic Diagnosis. Primary. Acute necrotizing arteriolitis of kidneys; pigmentation, thickening and desquamation of skin of face and scalp; fibrous pericardial, pleural and peritoneal adhesions; cardiac hypertrophy; subcutaneous and cerebral edema; hydrothorax (bilateral); ascites; focal pneumonia (right upper lobe); pigmentation and hyperplasia of axillary and abdominal lymph nodes. *Subsidiary.* Fibrous thickening of mitral valve; ovarian cyst. (The anatomic findings were consistent with the clinical diagnosis of disseminated lupus erythematosus.)

Summary and Conclusion

This study was undertaken in an effort to determine the rôle of the lymph nodes in the syndrome of disseminated lupus erythematosus. A review was made of 277 reported cases, and 3 additional cases are reported in detail. The disease is most prevalent among white individuals between the ages of 11 and 40 years, with females predominating over males 3.5 to 1. Three Negroes, 1 Hawaiian of Japanese extraction, and 2 Puerto Ricans were included in the group. The lymph nodes were enlarged, either locally or generally, in 66.7 per cent of those cases in which they were specifically mentioned. The frequency of enlargement parallels the general incidence of the disease as to age and sex. The cervical group of nodes was enlarged most frequently, followed by the mesenteric, axillary, inguinal and retroperitoneal groups. The morphologic changes in the enlarged nodes are described in detail. The histologic picture is chiefly one of edema and engorgement, associated not infrequently with areas of necrosis. These features are considered suggestive of, but not specific for, the disease.

BIBLIOGRAPHY

Baehr, George; Klemperer, Paul, and Schifrin, Arthur. A diffuse disease of the peripheral circulation (usually associated with lupus erythematosus and endocarditis). *Tr. A. Am. Physicians,* 1935, **50,** 139–155.

Banks, B. M. Is there a common denominator in scleroderma, dermatomyositis, disseminated lupus erythematosus, the Libman-Sacks syndrome and polyarteritis nodosa? *New England J. Med.,* 1941, **225,** 433–444.

Cabot Case No. 24201. Acute disseminated lupus erythematosus. *New England J. Med.,* 1938, **218,** 838–843.

Clarke, F. B., and Warnock, A. W. Lupus erythematosus acutus disseminatus. Report of a fatal case. *California & West. Med.*, 1926, **24**, 354–357.

Denzer, B. S., and Blumenthal, Sidney. Acute lupus erythematosus disseminatus. *Am. J. Dis. Child.*, 1937, **53**, 525–540.

Ehrmann, S., and Falkenstein, Fritz. Über Lupus erythematodes. *Arch. f. Dermat. u. Syph.*, 1922, **141**, 408–506.

Friedberg, C. K., and Gross, Louis. Nonbacterial thrombotic endocarditis associated with acute thrombocytopenic purpura. *Arch. Int. Med.*, 1936, **58**, 641–661.

Gall, E. A., and Stout, H. A. The histological lesion in lymph nodes in infectious mononucleosis. *Am. J. Path.*, 1940, **16**, 433–448.

Gennerich, Wilhelm. Über die Ätiologie des Lupus erythematodes. *Arch. f. Dermat. u. Syph.*, 1921, **135**, 184–207.

Gibson, Robert. Fatal case of lupus erythematosus, with post-mortem. *Brit. J. Dermat.*, 1925, **37**, 232–233.

Ginzler, A. M., and Fox, T. T. Disseminated lupus erythematosus: a cutaneous manifestation of a systemic disease (Libman-Sacks). Report of a case. *Arch. Int. Med.*, 1940, **65**, 26–50.

Hardaway, W. A. A case of lupus erythematosus presenting unusual complications. *J. Cutan. Dis.*, 1889, **7**, 447–450.

Hardaway, W. A. A case of lupus erythematosus presenting unusual complications. *J. Cutan. Dis.*, 1892, **10**, 268–272.

Kaposi, M. K. Neue Beiträge zur Kenntniss des Lupus erythematosus. *Arch. f. Dermat. u. Syph.*, 1872, **4**, 36–78.

Keefer, C. S., and Felty, A. R. Acute disseminated lupus erythematosus. Report of three fatal cases. *Bull. Johns Hopkins Hosp.*, 1924, **35**, 294–304.

Keil, Harry. Relation between "systemic" lupus erythematosus and a peculiar form of thrombocytopenic purpura. *Brit. J. Dermat.*, 1937, **49**, 221–237.

Keil, Harry. Conception of lupus erythematosus and its morphologic variants, with particular reference to "systemic" lupus erythematosus. *Arch. Dermat. & Syph.*, 1937, **36**, 729–757.

Keil, Harry. Dermatomyositis and systemic lupus erythematosus. I. A clinical report of "transitional" cases, with a consideration of lead as a possible etiologic factor. *Arch. Int. Med.*, 1940, **66**, 109–139.

Keil, Harry. Dermatomyositis and systemic lupus erythematosus. II. A comparative study of the essential clinicopathologic features. *Arch. Int. Med.*, 1940, **66**, 339–383.

Keith, N. M., and Rowntree, L. G. A study of the renal complications of disseminated lupus erythematosus: report of four cases. *Tr. A. Am. Physicians*, 1922, **37**, 487–502.

Klemperer, Paul; Pollack, A. D., and Baehr, George. Pathology of disseminated lupus erythematosus. *Arch. Path.*, 1941, **32**, 569–631.

Low, R. C., and Rutherford, Andrew. Post-mortem report on a case of lupus erythematosus. *Brit. J. Dermat.*, 1920, **32**, 326–330.

Lyon, J. M. Acute lupus erythematosus. *Am. J. Dis. Child.*, 1933, **45**, 572–583.

Madden, J. F. Acute disseminated lupus erythematosus. *Arch. Dermat. & Syph.*, 1932, **25**, 854–875.

Roberts, Leslie. Acute lupus erythematosus (aigu d'emblée). *Brit. J. Dermat.*, 1911, **23**, 167–178.

Rose, Edward, and Pillsbury, D. M. Acute disseminated lupus erythematosus—a systemic disease. *Ann. Int. Med.*, 1939, **12**, 951–963.

Sequeira, J. H., and Balean, H. Lupus erythematosus: a clinical study of seventy-one cases. *Brit. J. Dermat.*, 1902, **14**, 367–387.

Short, T. S. Fatal case of acute lupus erythematosus. *Brit. J. Dermat.*, 1907, **19**, 271–274.

Sibley, W. K., and Wynn, W. H. A fatal case of lupus erythematosus disseminatus. *Brit. J. Dermat.*, 1923, **35**, 323–324.

Spiethoff, B. Zur Ätiologie und Pathologie des Lupus erythematodes chron. und acut. Mitteilung über Bakterien- und Blutbefunde. *Arch. f. Dermat. u. Syph.*, 1912, **113**, 1047–1060.

Spiethoff, B. Das Blutbild bei chronischen und akuten Form des Lupus erythematodes. *Arch. f. Dermat. u. Syph.*, 1915–16, **121**, 269–277.

Templeton, H. J. Thrombopenia in acute disseminated lupus erythematosus. *Arch. Dermat. & Syph.*, 1934, **29**, 700–702.

[*Illustrations follow*]

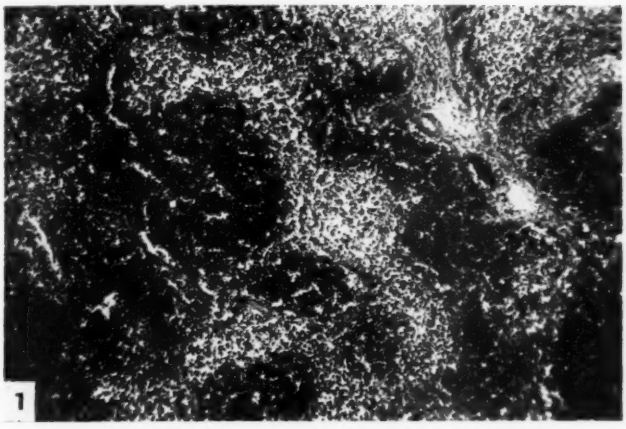
DESCRIPTION OF PLATES

PLATE 10

FIG. 1. Marked engorgement and loss of normal architecture in the enlarged lymph nodes. Hematoxylin and eosin stain. × 85.

FIG. 2. Edema of enlarged lymph nodes. Hematoxylin and eosin stain. × 250.

FIG. 3. Loss of normal architecture and cellular hyperplasia in enlarged lymph nodes. Hematoxylin and eosin stain. × 85.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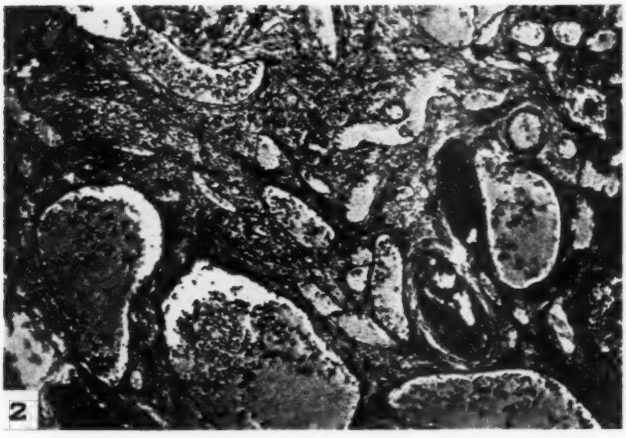
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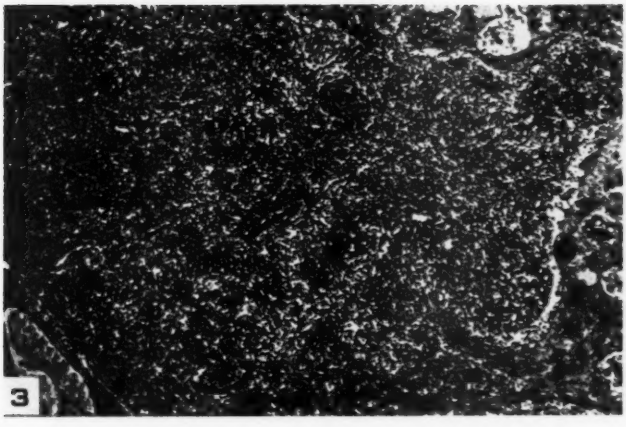
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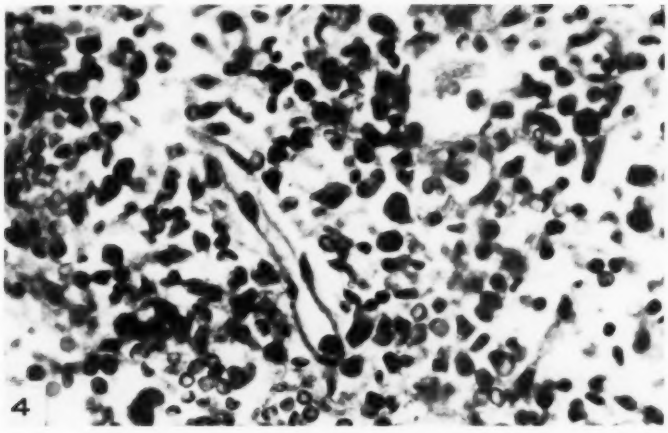
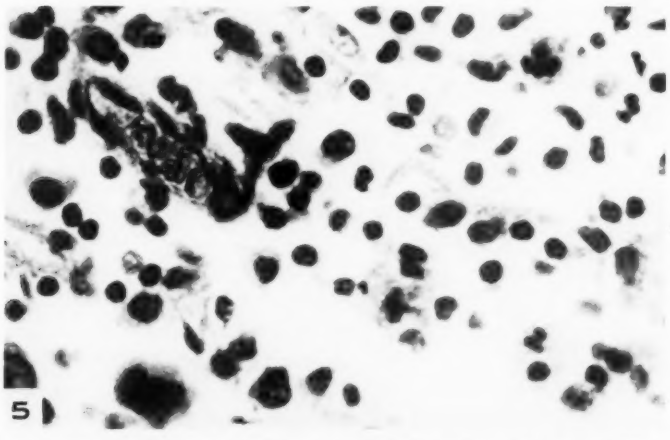
PLATE 11

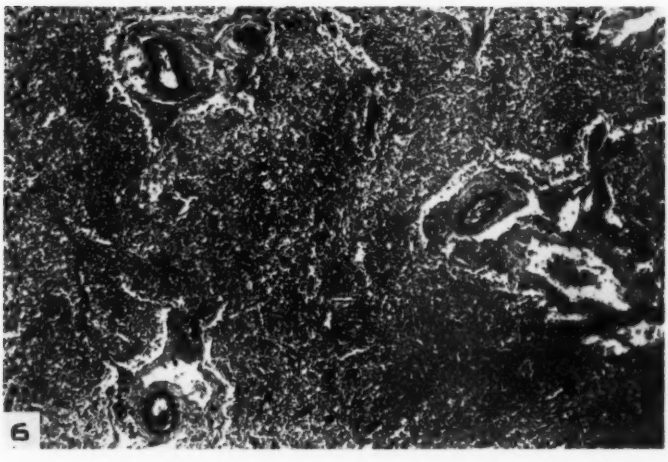
FIG. 4. Numerous plasma cells, one of which has two nuclei. Hematoxylin and eosin stain. × 700.

FIG. 5. Large cells with multilobed nuclei (megakaryocytes). Hematoxylin and eosin stain. × 775.

FIG. 6. Small arteries with collars of connective tissue. Masson's trichrome stain. × 40.







Fox and Rosahn Lymph Nodes in Lupus Erythematosus

MEDIASTINAL SYMPATHOGONIOMA*

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A concise classification of tumors of the sympathetic nervous system based on their embryogenesis has been proposed by Bielschowsky.[1] The most immature formative elements of this order, the sympathogonia, show no neurofibrils. They have small, round, dark-staining nuclei enveloped in a delicate, homogeneous, and often indiscernible cytoplasm. These primitive structures are multipotential and give rise to both sympathetic ganglion and chromaffin cells. In the latter case they pass through the stage of pheochromoblast and pheochromocyte formation. In their development into sympathetic ganglion cells the first stage of ripening is the sympathoblast, a larger cell with a vesicular nucleus and rather abundant elongated protoplasmic processes. The end-state of maturation yields large multipolar forms with intracellular fibrils and Nissl substance—the sympathetic ganglion cells. Corresponding to each of these developmental steps are neoplasms composed of analogous histologic types. The cells in the most primitive tumor, the sympathogonioma, retain the lymphocytoid character of the parent stem cell though their arrangement may follow several patterns. Sympathoblastomas, derived from the ensuing subdivision, are made up of large, irregular, pear-shaped or oval cells with cytoplasmic streamers. The ganglioneuromas are characterized by large sympathetic ganglion cells incorporated in their fibrillar structure. In utilizing such a classification it must be borne in mind that these growths frequently are not composed purely of cells belonging to a single analogous plane of maturation but that considerable overlapping occurs. In evaluating the histologic picture the diagnosis must frequently be based on the predominating cell type.

Sympathetic tumors of all types are widely distributed throughout the body. In addition to their rather frequent appearance in the medulla of the adrenal glands, they have been reported as occurring in the abdominal, cervical, thoracic and pelvic sympathetics, the jejunum, celiac ganglion, mesentery, coccygeal gland, liver, uterus, cavity of the nose, skin or subcutaneum, and scapular region.[2] There appears to be adequate ground for the concept that peripherally mi-

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TABLE I
Intrathoracic Tumors Having Origin in the Sympathetic Nervous System

No.	Date	Author	Age	Sex	Location	Gross appearance	Metastasis or extension	Microscopic diagnosis
1	1870	Loretz[3]	35 yrs.	F	Retropleural; lateral to left 2nd and 3rd thoracic vertebrae and adherent to 2nd and 3rd dorsal vertebral bodies	7.5 by 4.5 cm. in diameter; on section outer portion firm and soft; resembled uterine tissue	Extended into intervertebral foramina and the vertebral bodies	Ganglioneuroma
2	1897	Borst[4]			Retropleural; close to vertebral column	Almost size of two fists		Ganglioneuroma
3	1912	Friedrich[5]	73 yrs.	F	Retropleural; between right 6th and 8th ribs and corresponding dorsal vertebral bodies	7 by 2.8 cm.; rather firm; cut section gray-yellow with fine fibrillar structure	None	Nonmyelinated ganglioneuroma
4	1923	Anderson and Shennan[6]	12 wks.	F	Retropleural; region of right lung apex; attached to 2nd and 3rd ribs and sides of dorsal vertebrae	Ovoid, soft spongy mass 4.2 by 2.8 cm.; cut surface brownish pink, suggesting normal thyroid gland but darker	None	Neuroblastoma (small lymphoid cells and larger sympathoblasts; rosette formation)
5	1924	Miller[7]	39 yrs.	F	Retropleural; paravertebral at level of right 6th rib	Twice the size of a chestnut; weight 32 gm.; cut section ochre-yellow with occasional blood-red area; consistency spleenlike	None	Paraganglioma
6	1927	Cushing and Wolbach[8]	2 yrs.	M	Paravertebral; springing from lamina or transverse process of 6th dorsal vertebra	"Plainly palpable and semifluctuant"	Extraspinal-dural extension through intervertebral foramen	Biopsy of original tumor, sympathicoblastoma; at operation 10 years later, original tumor had disappeared; remaining extradural extension, a ganglioneuroma

7	1927	Capaldi[9] (Case 1)	44 yrs.	F	First tumor lateral to left C7 and D1 vertebrae, attached to 2nd rib below Second tumor on corresponding right side attached to first rib Third tumor beneath left adrenal	Size of pigeon's egg, firm, gray-white Bean-sized; similar appearance Size of small apple	Extension to spinal cord dura and into brachial plexus Extension to spinal cord dura	Neuroblastoma simplex (immature sympathoblastoma) Neuroblastoma simplex (immature sympathoblastoma) Neuroblastoma simplex (immature sympathoblastoma)
8	1927	Capaldi[9] (Case 2)	2 yrs.	F	Retropleural with massive extension into left upper thorax, cervical and axillary regions	Knobby, firm, gray-white tissue mass	Penetration of intervertebral foramina with extradural extension from D8 to medulla oblongata	Well differentiated ganglioneuroma
9	1932	Millar[10]	34 yrs.	M	Retropleural; adherent to left 6th, 7th, 8th thoracic vertebrae and penetrating left lung	On section, intensely black inner circular portion about 3 cm. in diameter surrounded by pinkish fleshy zone 6 cm. in diameter	Extension to lung, thoracic vertebral bodies and into spinal canal; metastases to ribs, skull, right adrenal, right pectoral muscle and right ear	Malignant melanotic ganglion cell tumor
10	1932	Scott and Palmer[2]	22 mos.	M	Retropleural; posterior to lower lobe of left lung	Lobulated, encapsulated mass 13 by 13 by 7.5 cm., weighing 572 gm.; cut section finely granular	Extension to right pleural cavity and extradurally over spinal cord	Sympathicoblastoma (sympathogonia and sympathoblasts; rosette formation)

TABLE I *continued*

No.	Date	Author	Age	Sex	Location	Gross appearance	Metastasis or extension	Microscopic diagnosis
11	1936	Frost and Wolpaw[11]	38 yrs.	M	Retropleural at apex of right thorax with extension into upper lobe of lung	9 by 5.5 by 7 cm.; irregularly nodular and not encapsulated; cut section dense, gray with somewhat whorled appearance	Extension into esophagus, right lung, last cervical vertebra and first 3 ribs; metastasis to right adrenal, kidney, jejunum, axillary nodes and subcutaneous tissue	Sympathoblastoma
12	1937	Stern and Newns[12]	7 yrs.	F	Posterior mediastinum with forward displacement of aorta and lobe of right lung	Not given	Extension to right lower lobe of lung and infiltration of the epidural space of spinal cord; bilateral pleural metastases	"Sympathogonioblastoma" (mixed sympathogonia and sympathoblasts)
13	1940	Philips[13]	39 yrs.	M	"Apex of left pleural cavity not attached to lung"	Size of plum, easily shelled out; on section alternating firm white, and soft hemorrhagic purple areas	None	Pheochromocytoma

grating primitive nerve cells of the developing sympathetic nervous system may come to rest almost anywhere in the body and are capable of proliferating and forming tumors at a later date. Table I briefly summarizes some of the salient features of 13 well documented sympathetic tumors recorded as occurring within the thorax.

The tumor to be described is of singular interest because of its peculiar location in the anterior mediastinum, and its striking histologic composition. The occurrence in a patient of advanced age and the biologic behavior of a growth of such a primitive type are also very unusual features.

Report of Case

M. C., a colored female, 65 years of age, was brought by ambulance to the accident room of the Cincinnati General Hospital on November 20, 1940, in a semicomatose state. According to the patient's son, 4 days before admission she had complained of severe headache and dizziness, accompanied by progressive general weakness. Chills, fever, vomiting, or other associated symptoms had not been observed. The patient became drowsy and stuporous shortly before admission. It was learned that during the past 2 years there had been occasional attacks of shortness of breath and precordial pain. A year before, she had been confined to bed because of congestive heart failure and at the time exhibited considerable swelling of her legs and abdomen. After 2 months she had recovered sufficiently to be able to perform housework with only moderate dyspnea and had been in fair health until the onset of her present illness. No other relevant facts were obtained from the history. Physical examination on admission showed a semicomatose, elderly Negress, who responded incoherently and indifferently to questioning. Temperature, 101.5° F.; pulse, 114; respiration, 34; blood pressure, 185/108. Her neck was rigid in all directions. Kernig's and Babinski's signs were positive. Deep reflexes were hypoactive in the upper extremities. Knee jerks were absent in both lower extremities. Consolidation was present at the base of the left lung, posteriorly. A spinal puncture was performed and a small amount of milky fluid under increased pressure was removed. The white blood cell count of the spinal fluid was 17,600 with 98 per cent polymorphonuclear neutrophils. *Haemophilus influenzae* was found on smear and culture. Blood culture was also positive for *H. influenzae*. The blood Kahn was negative. Sulfapyridine therapy was instituted but proved to be ineffectual and the patient succumbed 2 days after admission.

Autopsy Findings. An abstract of the positive findings at autopsy, performed 18 hours after death, follows:

The patient was a moderately obese, colored female appearing the stated age of 65 years. Herpetic lesions were present on both lips. The upper jaw was edentulous and the remaining lower teeth carious. There was bilateral arcus senilis. A few fibrous adhesions bound the apex of the right lung and the diaphragmatic surfaces of both lower lobes to the parietal pleurae. The medial half of the right transverse pulmonary fissure was incompletely developed and the oblique fissure posteriorly on the same side was absent. Emphysematous bullae were present over the anterior margin of the right middle lobe. The left

lower lobe was increased in consistency and had a dark red, moist, slightly granular appearance on section. The remaining lobes were dry, inelastic and hypercrepitant except in the dependent portion where they were moist and poorly aërated. Projecting into the anterior superior mediastinum, overlying the superior vena cava and attached laterally by adhesions to the first portion of the aorta, was a round, firm mass, 5 cm. in diameter. This was covered by the pericardial sac whose upper portion was bound rather firmly to the anterior and lateral surfaces of the tumor by old fibrous adhesions. Easily broken, fine, white fibrous bands fastened the adherent pericardium to the mediastinal surface of the right lung. On stripping away the pericardium, the tumor was found to be well encapsulated and rather loosely attached by fibrous adhesions to the anterior surface of the superior vena cava and the upper portion of the markedly dilated right atrium, lying above and some distance anteriorly to the right pulmonary artery and eparterial bronchus (Fig. 1). No nerve fibers or ganglia were seen entering its capsule. The cut surface of the tumor was uniformly reddish brown; its consistency soft. Elsewhere both layers of the pericardial sac were lightly bound together by delicate fibrous strands. Both cardiac ventricles were slightly dilated. The musculature was uniformly pale reddish brown and soft, left ventricular wall thickened and the endocardial surfaces of all the chambers were smooth and glistening. Raised yellow intimal plaques were present throughout the aorta. In the region of the arch these were partially calcified. The abdominal organs were in normal anatomic relation to one another. Eight small black calculi were present in the fundus of the gallbladder. The gallbladder wall was thin and pliable. The adrenal glands were intact. A circumscribed, firm, myomatous tumor within the wall of the uterine fundus measured 3 cm. in diameter. Several soft red polyps were present in the uterine cavity. A round, multilocular serous cyst, 8 cm. in diameter, occupied the left ovary.

The brain weighed 1275 gm. A thick, greenish, fibrinopurulent exudate distended the subarachnoid space over the convex surfaces of both cerebral and cerebellar hemispheres and collected at the base of the brain in the region of the optic chiasm. A similar exudate involved the leptomeninges of the spinal cord.

Microscopic Findings. Numerous sections taken throughout the mediastinal tumor showed a strikingly uniform cell type and composition. The dense, enveloping, fibrous connective tissue capsule contained focal deposits of calcium salts. One section cut near the posterior surface showed a few nests of tumor cells penetrating the

capsule and infiltrating an adherent tag of adipose tissue. No other areas of capsule invasion were found. Stained with hematoxylin and eosin, the tumor cells in practically all sections appeared to consist entirely of nuclei. These were predominantly round or oval, rich in chromatin and ranged in size from one to two times the diameter of a red blood cell. Stained with Masson's trichrome stain, however, a delicate enveloping rim of vermilion-red cytoplasm could be demonstrated about most of the prominent purple-black nuclei. No cytoplasmic granules were seen in Zenker's fluid-fixed material. In some instances the cytoplasm assumed a bipolar arrangement, streaking out at each end of the nucleus in a wedge-shaped pattern for a distance equal to or surpassing the greatest diameter of the nucleus (Fig. 2). Others showed the base of the cytoplasmic wedge resting on only one pole of the nucleus, thence abruptly narrowing to an elongated fine thread which was attached to an adjacent cell border or a blood vessel. While the tumor possessed a rather rich capillary network, only rarely were such threadlike processes found approaching the vessel wall at right angles, the cells usually appearing parallel to the vessel axis (Fig. 3). The modification by Foot and Foot of Bielschowsky's silver stain showed the cell nuclei to be intensely argentophilic, but only very rarely and by repeated sectioning could a reticulum fiber be located. Intracellular or extracellular neurofibrils could not be demonstrated with Bodian's protargol stain. The majority of the cells were of the small, round, lymphocytoid type (Fig. 4), and there were but few attempts at rosette formation; where present, these lacked the circular arrangement of the cells about a central limiting membrane considered by Bailey and Cushing [14] typical of true rosettes (Figs. 5 and 6). An occasional field showed whorl-like masses constructed of closely packed, short, oval cells (Fig. 7). Under higher magnification some of these nuclei were slightly indented on their lateral surfaces or diminished in thickness toward one pole exhibiting a carrot shape suggesting the formation of lemmoblasts. Further support for assuming a deviation in this direction of growth was brought out in a rare zone in which there was an interlacing bundlelike arrangement of elongated spindle cells such as is frequently encountered in neurilemomas of peripheral nerves (Figs. 8 and 9). In sections stained with hematoxylin and van Gieson's mixture there were only a few isolated zones in which collagenous supporting fibers were found.

In addition to this neoplasm in the anterior mediastinum, examination of the remaining organs showed a chromophobe adenoma, measuring 7 by 6 mm. in the anterior lobe of the pituitary gland; a small mucosal adenomatous polyp of the fundus of the stomach; multiple

intramural leiomyomata of the fundus uteri; several adenomatous endometrial polypi, and a multilocular serous cyst of the right ovary.

Pathologic Diagnoses. The final pathologic diagnoses were: acute influenzal cerebrospinal meningitis; bronchopneumonia, lower lobe of the left lung; sympathogonioma of anterior mediastinum originating beneath the pericardium, probably from within the sympathetic rami of the deep cardiac plexus; chronic obliterative pericarditis; slight myocardial hypertrophy and dilatation; focal myocardial scarring; chronic pulmonary emphysema; pleural adhesions; chromophobe adenoma of the anterior lobe of the pituitary gland; adenomatous polyp of the gastric mucosa; multiple intramural leiomyomata of the uterus; endometrial polypi; multilocular serous cyst of the right ovary; cholelithiasis; chronic cholecystitis; aortic atherosclerosis with focal calcification; moderate generalized arteriosclerosis.

Discussion

Among the intrathoracic sympathetic tumors listed in Table I, two examples are of the chromaffin cell type, the remaining falling under the heading of sympathetic tumors in the strict sense of the word. In almost all of the latter the primary site was behind the parietal pleura with growth anteriorly into the thoracic viscera. In many, direct continuity with the sympathetic chain was anatomically demonstrable. None of these tumors exhibited either a location or histologic structure comparable to that in the case presented. In this tumor, the uniform cell plan observed throughout the numerous sections leaves little doubt of its proper classification according to Bielschowsky's [1] outline. Absence of maturation into larger sympathoblastic cells further indicates its embryonal character, although the presence of occasional scattered small cells with unipolar and bipolar cytoplasmic processes may represent an intermediate step in this direction. A few areas which contain carrot-shaped forms suggest the production of lemmoblasts, and the occasional interlacing bundles of elongated spindle cells are in keeping with a later development along this line. Earlier investigations on the embryologic development of the sympathetic system in mammals corroborate such a formation of Schwann cells from the immature anlagen of sympathetic ganglia.[15] The location of the tumor beneath the pericardium over the right atrium indicates a probable derivation from embryonal sympathetic elements contained in the deep cardiac plexus.

The complete encapsulation of the tumor with only one microscopic field displaying penetration of tumor cells into the surrounding tissue, together with the absence of distant metastases, seems to belie the

rapid growth which such immature cell types would indicate. Similar growths appearing elsewhere in the body are usually characterized by early and widespread metastases. Why such a protracted growth rate is present in this case is not clear, and certainly the anatomic location of the tumor could not influence its capacity to grow. The appearance of such a tumor in a patient in the sixth decade of life is also unusual, though poorly differentiated sympathetic neoplasms in other locations have been described as occurring in late adult life.[16, 17] One can readily understand how more widely disseminated neoplasms of the mediastinum and lungs, designated as lymphosarcomas, thymomas or small-cell carcinomas, might be confused with embryonal tumors of the sympathetic nervous system in this location, once growth and dissemination had become active.

REFERENCES

1. Bielschowsky, Max. Neuroblastic Tumors of the Sympathetic Nervous System. In: Penfield, Wilder. Cytology and Cellular Pathology of the Nervous System. Paul B. Hoeber, Inc., New York, 1932, **3**, 1085–1094.
2. Scott, Ernest, and Palmer, D. M. Intrathoracic sympatheticoblastoma. Report of a case. *Am. J. Cancer*, 1932, **16**, 903–917.
3. Loretz, Wilh. Ein Fall von gangliösem Neurom (Gangliom). *Virchows Arch. f. path. Anat.*, 1870, **49**, 435–437.
4. Borst, M. Demonstration eines wahren Neuroms. *Klin. Wchnschr.*, 1897, **34**, 1062–1063.
5. Friedrich, Jakob. Ein Fall von Ganglioneurom der Sympathikus. Gleichzeitig ein Beitrag zur Theorie der autogenen Entstehung der Nervenfasern. *Frankfort. Ztschr. f. Path.*, 1912, **10**, 456–473.
6. Anderson, J. S., and Shennan, T. A neuroblastoma of the thoracic cavity. *J. Path. & Bact.*, 1923, **26**, 545–546.
7. Miller, J. W. Ein Paragangliom des Brustsympathicus. *Centralbl. f. allg. Path. u. path. Anat.*, 1924–25, **35**, 85–94.
8. Cushing, Harvey, and Wolbach, S. B. The transformation of a malignant paravertebral sympathicoblastoma into a benign ganglioneuroma. *Am. J. Path.*, 1927, **3**, 203–216.
9. Capaldi, Benvenuto. Zwei Fälle von Sympathikoblastom. *Frankfort. Ztschr. f. Path.*, 1927, **35**, 83–100.
10. Millar, W. G. Malignant melanotic tumour of ganglion cells arising from thoracic sympathetic ganglion. *J. Path. & Bact.*, 1932, **35**, 351–357.
11. Frost, T. T., and Wolpaw, S. E. Intrathoracic sympathoblastoma producing the symptomatology of a superior pulmonary sulcus tumor (Pancoast). *Am. J. Cancer*, 1936, **26**, 483–492.
12. Stern, R. O., and Newns, G. H. Tumours of the sympathetic nervous system in children; study of 25 cases. *Arch. Dis. Childhood*, 1937, **12**, 267–290.
13. Philips, Benjamin. Intrathoracic pheochromocytoma. *Arch. Path.*, 1940, **30**, 916–921.

14. Bailey, Percival, and Cushing, Harvey. A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis. J. B. Lippincott Company, Philadelphia, 1926.

15. Kohn, Alfred. Ueber die Entwickelung des peripheren Nervensystems. *Anat. Anz.*, 1905, suppl. **27**, 145–150.

16. Ritter, S. A. Neuroblastoma of the small intestine. *Am. J. Surg.*, 1938, **41**, 486–493.

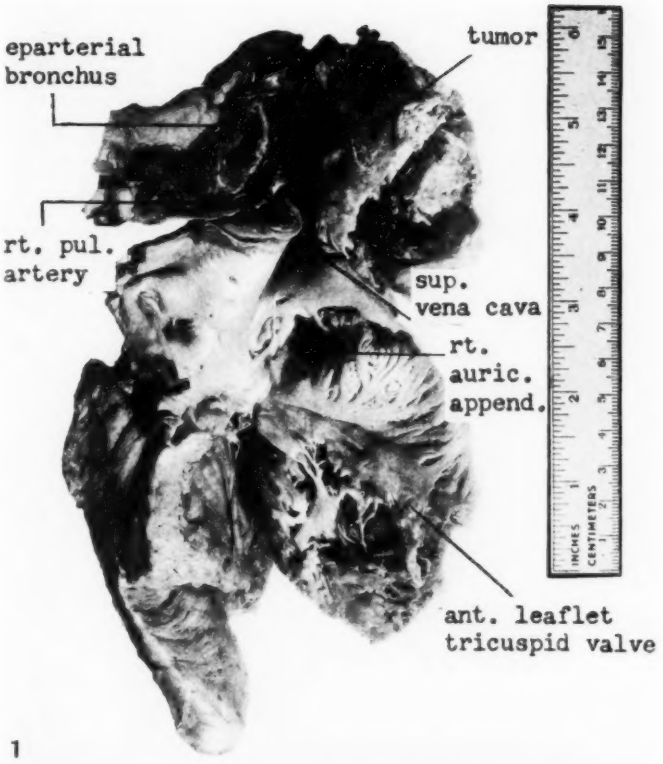
17. Meltzer, Sara. Neuroblastoma occurring in adults. *Canad. M. A. J.*, 1926, **16**, 647–651.

DESCRIPTION OF PLATES

Plate 12

Fig. 1. Relation of tumor to opened right atrium and associated structures.

Fig. 2. Bipolar arrangement of cytoplasm about oval nucleus. Masson's trichrome stain. × 1125.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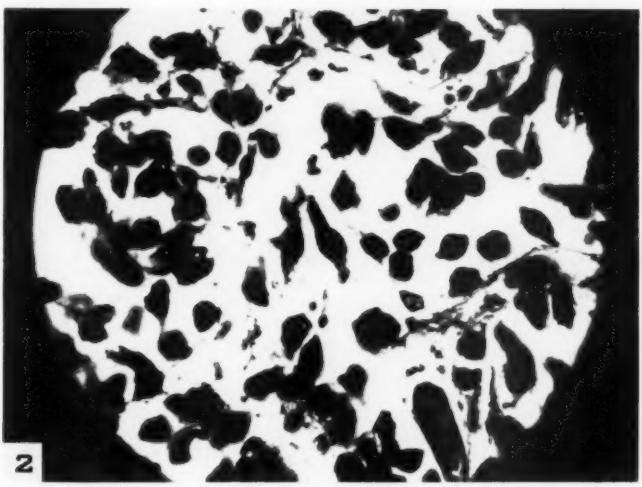
tumor
rt. pul.
artery
sup.
vena cava
rt.
auric.
append.
ant. leaflet
tricuspid valve
INCHES
CENTIMETERS

1

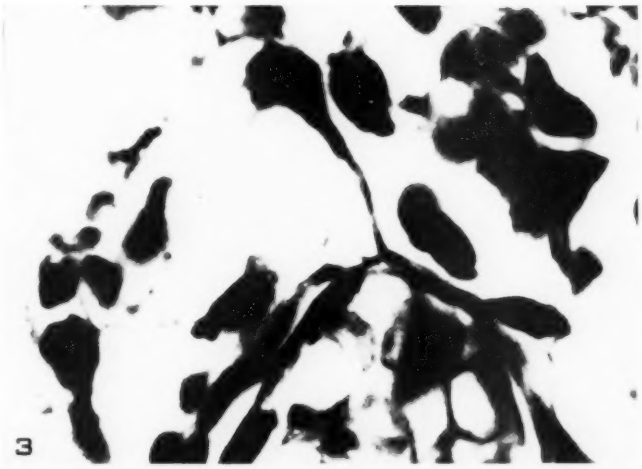


2

PLATE 13

FIG. 3. Filamentous cytoplasmic streamer attached to capillary wall. Masson's trichrome stain. × 2250.

FIG. 4. Compact area of uniformly round cells. Hematoxylin and eosin stain. × 160.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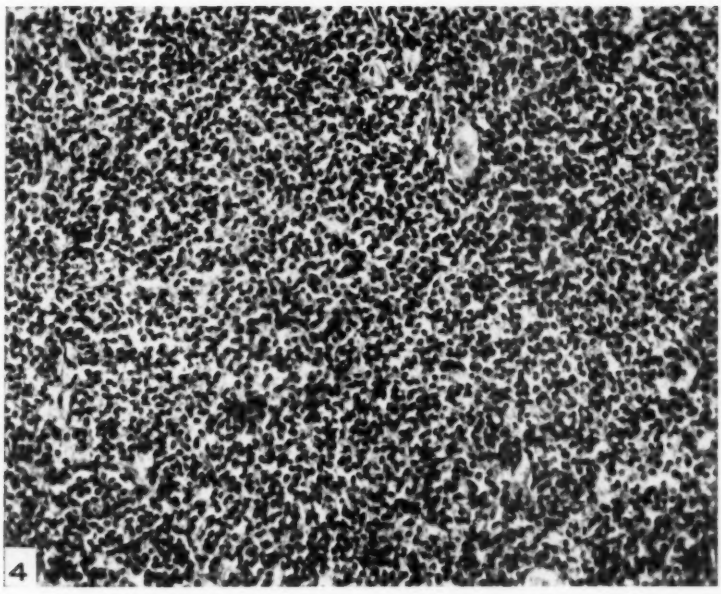
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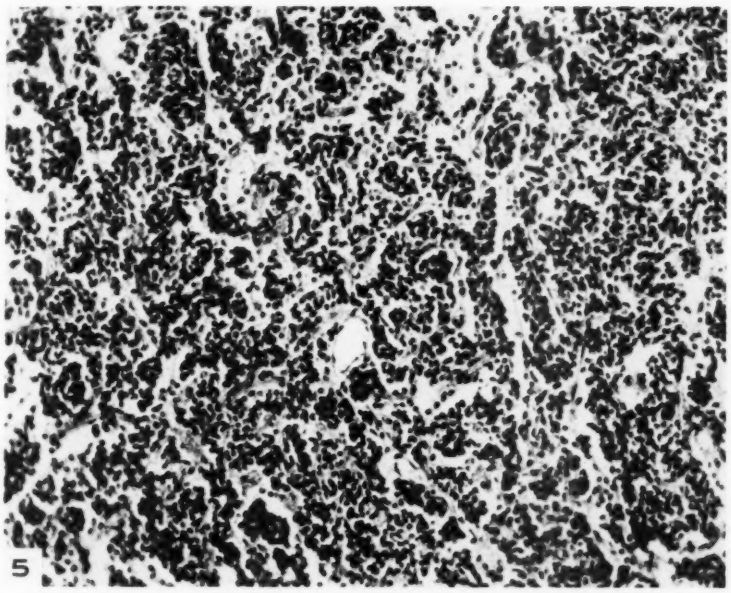
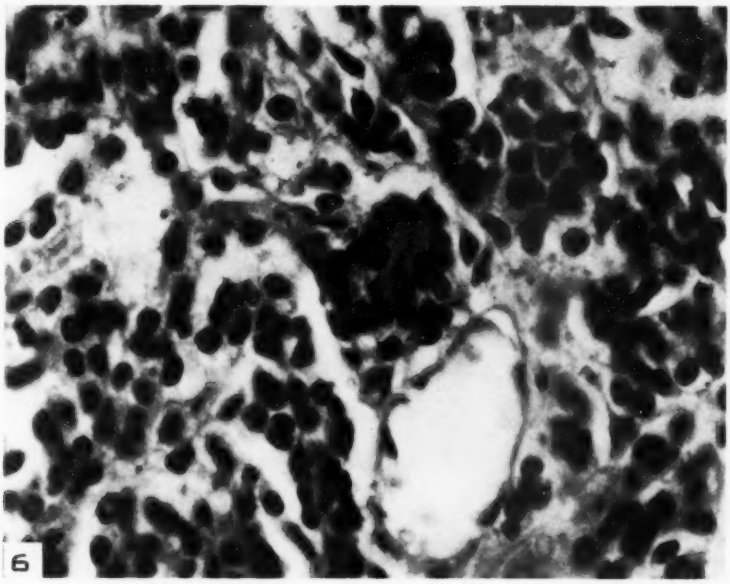
PLATE 14

FIG. 5. Areas showing pseudorosette formation. Hematoxylin and eosin stain. × 160.

FIG. 6. Areas showing pseudorosette formation. Hematoxylin and eosin stain. × 600.



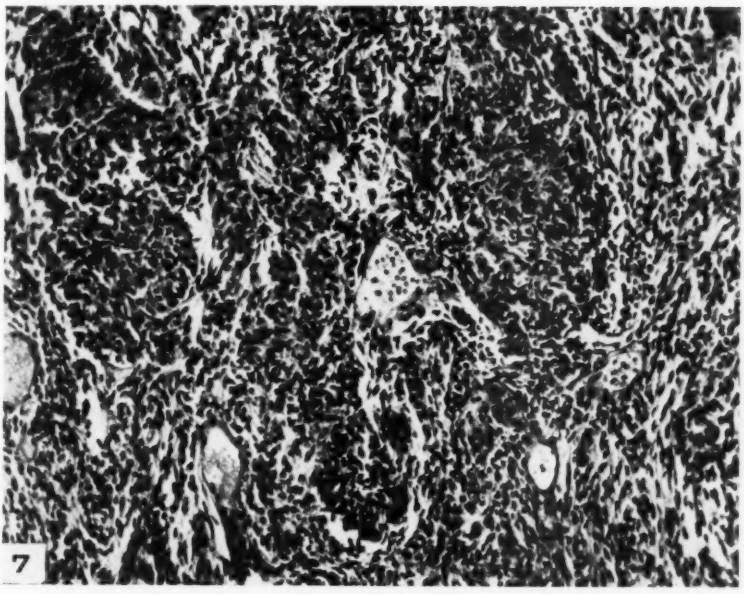


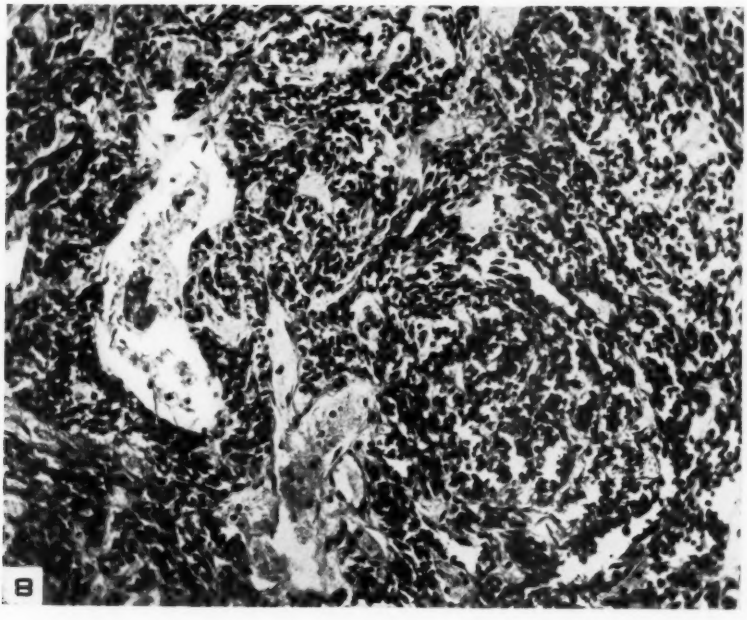
Sailer Mediastinal Sympathogonioma

PLATE 15

FIG. 7. Rounded masses of short, oval and carrot-shaped cells. Hematoxylin and eosin stain. × 160.

FIG. 8. Interlacing bundles of elongated spindle cells. Hematoxylin and eosin stain. × 160.

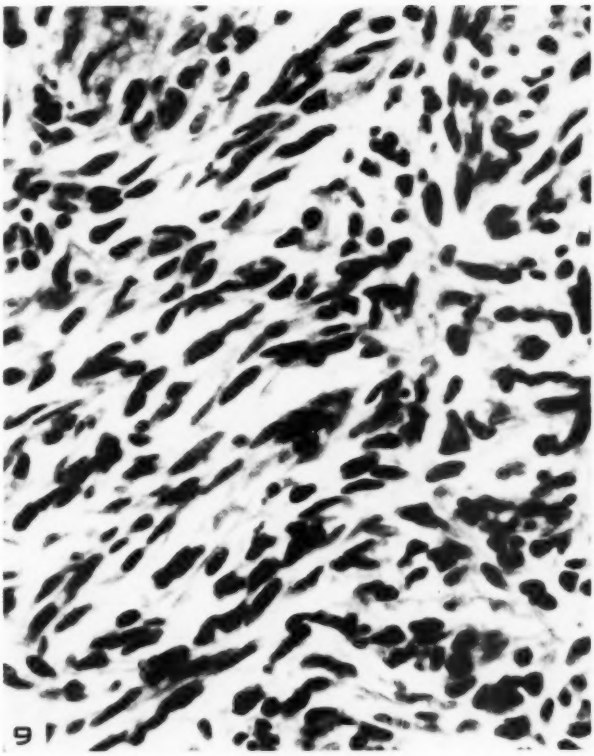




Sailer Mediastinal Sympathogonioma

PLATE 16

FIG. 9. Interlacing bundles of elongated spindle cells. Hematoxylin and eosin stain. × 600.



Saller Mediastinal Sympathogonioma

MEDIAL HYPERTROPHY OF THE RENAL ARTERIOLES IN PREGNANCY *

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In the recent European literature much attention has been given to the special mode of termination of renal afferent arterioles in man and many other mammals. Smith [1] in his Harvey Lecture has reviewed the historical development of our knowledge of this subject and the reader may get fuller details there. For purposes of this report, it may suffice to summarize this literature as follows: A number of observers (Clara,[2] Becher,[3] Goormaghtigh,[4-6] Benninghoff [7]) have stressed the occurrence of nonfibrillar, "clear" smooth muscle cells in the media of normal vessels, and some [4, 6, 8, 9] have described granular chromophilic cells in the termination of the afferent arterioles in some species. Some regard them as related modifications, and both types as modified smooth muscle cells. Zimmermann [10] described, as well, the peculiar expansion of the terminal portion of the afferent arteriole due to an increase in medial cells, which he designated as the "Polkissen" (polar pad). This may be eccentric or concentric. Goormaghtigh has designated these groups of cells as the "juxtaglomerular apparatus." He [11-13] has recently claimed that in renal disease in man and in experimental renal hypertension in dogs and rabbits the clear cells and granular cells multiply and undergo hypertrophy. To them he has assigned an endocrine rôle in the formation of the pressor substances probably responsible for renal hypertension. Dunihue and Candon [14] have confirmed these claims in rabbits.

A nephrectomy performed for the relief of intermittent hematuria of at least 3 years' duration in a colored female, 29 years old, revealed unusual vascular lesions of the kidney. Because of the remarkable transformation of the media of the afferent arterioles and the enlargement of the "Polkissen" encountered in the kidney in the absence of hypertension and during pregnancy, this case seems worthy of publication. The subsequent development of eclampsia and mild hypertension lend special interest to the vascular changes to be described.

Report of Case

E. M., a colored female, 29 years of age, was first admitted to the Urological Service of Bellevue Hospital in April, 1938. At that time she complained of painless hematuria of 1 year's duration. There had been a slight loss of weight (8 lbs.). There were no other complaints. She reported that she had been treated at another

* Received for publication, April 13, 1942.

hospital for the same complaint 3 months before entering Bellevue Hospital. At unstated periods she had previously received injections for syphilis, and in 1932 she had had a spontaneous miscarriage.

Physical examination revealed no signs of organic disease. The patient did not appear to be ill. Some observers thought there was an indefinite mass in the left upper quadrant, possibly the spleen or kidney. The blood pressure on this admission was 116/68 mm. Hg. There was moderate anemia with 45 per cent hemoglobin, 3.4 million erythrocytes per cmm., 4,000 leukocytes per cmm., with 81 per cent polymorphonuclear leukocytes. Blood nonprotein nitrogen was 34 mg. per 100 cc. Cystoscopy revealed a normal bladder, but blood could be seen trickling from the left ureteral orifice. Intravenous pyelography revealed no abnormalities; there was no record of urine culture. A left-sided abscess found in Bartholin's gland was incised. Culture of the pus yielded a staphylococcus, Gram-positive bacilli and diphtheroids.

With these findings, some urologists believed that hematuria might have been the result of infection and inflammatory changes, and the patient was given urinary antiseptic drugs. Following the administration of iron by mouth, the erythrocytes rose to 5 million per cmm. and the hemoglobin to 70 per cent.

However, the hematuria continued. The patient was readmitted in August and again in October, 1938, and the same findings were reported. Cystoscopic and pyelographic investigations were repeated with the same results. Intravenous injection of methylene blue showed that the dye appeared in the left ureter a little earlier (5 minutes) than on the right side (8 minutes). No other renal function tests were done. Blood nonprotein nitrogen was within normal limits.

The fifth and sixth admissions occurred in November and December, 1939, and at this time the patient was pregnant. In the latter admission she was estimated to be in the third month of gestation. Urinary findings persisted as before. Cystoscopy was repeated and a tissue specimen of each ureteral orifice was taken and reported to show chronic inflammation. There was moderately severe anemia, and the patient was given two blood transfusions. Erythrocytes rose from 2.0 to 3.9 million per cmm. and the hemoglobin from 30 to 60 per cent.

Members of the Obstetrical Service believed that the pregnancy should not be interrupted. The urologists now considered nephrectomy advisable, for a neoplasm was believed to be the most probable cause of the hematuria. Consequently, on February 23, 1940, a left nephrectomy was performed and the patient made an uneventful recovery. Pregnancy was not disturbed. There was no further hematuria. The blood pressure before and for a week after operation ranged from 110 to 114 systolic and from 68 to 70 diastolic. The kidney will be described later in this report.

Postoperative and Antepartum Course. Special renal function tests were performed 2 weeks after operation and at intervals thereafter. They are referred to below. The blood pressure remained normal until May 13, 1940, when in the ninth month of gestation it rose to 140/96. The urine showed a trace of albumin which persisted for the next 2 weeks. At the patient's last visit to the Prenatal Clinic on May 28th the blood pressure was 130/78 and the urine was normal.

On the night of May 30, 1940, in the ninth month of gestation, she had two convulsive seizures at home and was taken to Harlem Hospital in a semistuporous condition. On physical examination slight edema of the ankles was noted and the blood pressure was found to be 140/90 mm. Hg. One hour after admission a spinal tap was done. Clear fluid, 35 cc. in amount, was removed under increased tension. There was another convulsion and the patient went into a deep coma. Chemical study of the blood showed creatinine, 1.4 mg. per cent; urea nitrogen, 14 mg. per cent; sugar, 120 mg. per cent, and uric acid, 10.5 mg. per cent. Urine obtained by catheter contained albumin, 4 plus; and microscopic examination showed many erythrocytes and occasional granular and hyaline casts.

Labor. Coma persisted and spontaneous labor began 16 hours after admission. The first stage lasted 14 hours, the second stage 25 minutes, and the patient spontaneously delivered a stillborn male child. During labor the blood pressure was 175/105 mm. Hg, the temperature was 104° F. and the pulse was 140; respiration numbered 26 per minute.

Puerperium. The patient came out of coma 16 hours after delivery and began to void large amounts of urine frequently.

Blood Pressure Readings During the First 10 Days Postpartum

1st day	140/90 mm. Hg	6th day	140/108 mm. Hg
2nd day	150/100 mm. Hg	7th day	152/110 mm. Hg
3rd day	130/106 mm. Hg	8th day	128/84 mm. Hg
4th day	132/108 mm. Hg	9th day	110/84 mm. Hg
5th day	144/114 mm. Hg	10th day	116/84 mm. Hg

A urinary concentration test was done on the twelfth day postpartum. Specific gravity ranged from 1.001 to 1.018. There was abundant volume in both the concentrated and dilute specimens. The Kahn test was done on the spinal fluid and was reported to be positive. Blood chemical determinations on the seventh and twelfth days postpartum showed creatinine, 1.3 mg. per cent; urea nitrogen, 13 and 10 mg. per cent respectively, and uric acid, 6 mg. per cent.

After discharge on the 13th day postpartum, the patient was referred to the Nephritis and Hypertension Clinic at the New York University College of Medicine. Arranged as Table I are significant laboratory findings obtained during her visits to the clinic.

TABLE I

Postpartum Observations in Nephritis and Hypertension Clinic at the New York University College of Medicine

Date	Blood pressure	Urine
6/26/40	134/90 to 160/100	
7/2/40	124/82	Sp. gr., 1.010; protein, trace; red blood cells, 100 to 150
8/13/40	114/80	
9/24/40	112/74	Sp. gr., 1.016; protein, faint trace; no red blood cells
10/15/40	126/80	Sp. gr., 1.020; no protein; no red blood cells
Nov., 1940	122/82	
Dec., 1940	130/90	
Jan., 1941	120/80	
Feb., 1941	130/82	
Mar., 1941	114/78	
May, 1941	116/80	No proteinuria or hematuria
June, 1941	130/86	Normal
Oct., 1941	126/90	Normal
Apr., 1942	120/80	Normal

There were no specific complaints on any visit.

Renal function was investigated * by methods applied by Smith, Goldring and Chasis.[15] Inulin clearance was used as a measure of the rate of glomerular filtration, diodrast clearance as a measure of the effective renal blood flow and diodrast Tm (tubular mass) to indicate the available functioning tubular tissue. At 5 months' gestation, which was 16 days after nephrectomy, the filtration rate and the effective renal blood flow were 75 per cent of normal, whereas the diodrast Tm was 50 per cent normal. (This was computed by comparison with average values in

* These tests were done, and will be reported in detail, by Drs. H. C. Taylor, Jr., Irwin Wellen and Catherine Welsh, of the Department of Obstetrics and Gynecology, New York University College of Medicine.

normal pregnant women.) These tests were repeated at 1, 5 and 9 months after delivery, the latter being 1 year after nephrectomy. The filtration rate was unchanged, the effective renal blood flow rose to normal level and the diodrast Tm increased to 80 per cent of the normal value. They represent, of course, the renal function of the remaining right kidney.

Pathologic Findings

The left kidney, operatively removed, appeared to be normal in size. The capsule stripped readily, leaving a somewhat pale, grayish pink surface. The organ was bisected in the longitudinal plane directed toward the pelvis. The cut surface revealed normal markings. The cortex was uniform in width and appearance. The pelvis was blood-stained and there were a few fresh clots adherent to the lining. On washing, tiny hemorrhages were visible in the pelvic lining and in the portion of the ureter attached to the pelvis.

Histologic Study. Blocks were fixed immediately in Helly's fluid, 10 per cent neutral formalin and Kopsch's fluid for mitochondria. Frozen sections were stained with Sudan IV for fat. Blocks were taken from all portions of the kidney and sections were cut in short strips composed of 16 to 20 serial sections, 5 μ in thickness. Alternating slides were stained by Masson's trichrome method (as modified by Goldner), and with azan-carmine. Some sections were stained by Mallory's method using phosphotungstic acid hematoxylin; others with van Gieson's picrofuchsin stain combined with Weigert's elastic tissue stain. Additional sections were stained for reticulum by the method of Foot and some were stained with Giemsa's stain. Tissue fixed for mitochondria was stained by the Altmann-Kull method.[16]

Sections from all parts of the kidney revealed uniform structure. The architecture was well preserved, but even at low magnification a striking alteration was visible in all the arterioles and a similar change could be traced backward in the medium-sized interlobular arteries. Except for the presence of a few red blood cells in some tubular lumina, no changes were found in the glomeruli or the tubules.

The vascular change consisted of hyperplasia and hypertrophy (Fig. 1) of the medial cells which thickened the walls of all the arterioles at the expense of the caliber of the lumina. In the terminal portions of the afferent arterioles this hyperplasia and hypertrophy produced small tumorlike swellings at their entrance into the glomeruli (Fig. 2). These correspond to the "Polkissen" which represent the normally thickened and expanded portion of the termination of the afferent arteriole as it enters the glomerulus. The hypertrophy of the terminal portions of the afferent arteriole in places indented the glomerulus. In other places it indented the neighboring tubules. The

lining endothelium appeared normal and there was no evidence of unusual distal extension or reduplication of the elastica in these arterioles.

Sections stained with hematoxylin and eosin gave only a slight suggestion of the degree of this change or of the swelling of the individual cells, but preparations stained by Masson's trichrome method or by azan-carmine revealed the details perfectly. They showed that none of the medial cells was fibrillar. With the azan-carmine stain they seemed quite clear, almost without cytoplasm, but under high magnification with slightly reduced illumination, a cytoplasmic ground substance could be seen.

In the intralobular arteries the media was similarly hypertrophied (Fig. 3), but there was also intimal and subintimal proliferation of tissue that was composed of young fibroblasts, smooth muscle cells and dense collagen. This proliferation served to narrow the lumen. There was no reduplication of the elastic membranes in these vessels. One interlobular artery contained a hyaline, sclerotic, intimal plaque which eccentrically narrowed the lumen. A small plaque was present in one interlobar branch, but most of the lobar branches of the renal arteries showed no sclerotic changes or intimal thickening.

Search for chromophilic granules like those that may be found in the terminal portion of the afferent arterioles of other mammalian kidneys was negative. Study of the efferent arteriole where it could be detected showed that similar swollen medial cells were present. These were seen to extend for only 15 to 20 μ along the efferent arterioles.

Sections of the pelvis and calyces revealed no inflammatory changes. In one section there was a small fresh hemorrhage without reaction just beneath the lining epithelium. In one place the epithelium had been freshly eroded. A similar process was seen in a ureteral section. In addition, where the lining cells were intact, marked swelling of the superficial cells was observed. The cytoplasm appeared vacuolated due to the presence of faintly basophilic mucoid material. However, this material did not give a positive stain for mucin.

Pathologic Diagnosis. Diffuse medial hyperplasia and hypertrophy of the afferent arterioles; focal fibromuscular intimal sclerosis with narrowing of interlobular renal arteries; multiple hemorrhagic erosions of the pelvis and ureter; hematuria.

Discussion

The changes detected in the kidney are unique in our experience and their counterpart has not been found thus far in a search of the literature. Jores,[17] in reviewing vascular disease, recognized the

occurrence of hypertrophy and hyperplasia of the media of small arteries. Recently, Castleman, Smithwick and Palmer [18] have studied biopsy material obtained from the kidneys of patients with very early stages of "essential hypertension." These observers report medial hypertrophy and other changes in 16 cases so studied. The possibility exists, therefore, that the lesion described here may be related to changes encountered in hypertension.

In unpublished studies of renal arterioles in experimental and human hypertension, nothing equal to it has been found in normal or ischemic kidneys of man or of several mammals, with or without hypertension. Other surgically-removed human kidneys, from males and nonpregnant females, similarly fixed, have not yielded such pictures of medial hypertrophy. This would exclude the mode of removal or time and manner of fixation as having any influence. Other kidneys obtained from patients dying in pregnancy or a few days postpartum have been equally free from such changes, but in none was fixation as prompt.

Since the patient did not have hypertension before nephrectomy or for some time after it, the morphologic changes found in the afferent arterioles can hardly be related to the patient's blood pressure. However, considering their universality, it seems likely that the same changes were present in the surviving contralateral kidney. If so, the removal of one kidney may have contributed to a lowering of the total blood flow due to narrowing of the vascular bed and to a reduction of normally functioning kidney tissue and of antipressor substances possibly produced by parts of one or both kidneys. Estimations of the renal blood flow in the surviving kidney were below normal for at least 9 months after contralateral nephrectomy. Such an imbalance might have precipitated hypertension of the renal type. Against this interpretation is the long interval (3 months) that elapsed before the onset of hypertension. Furthermore, it was mild and lasted only 6 to 8 weeks altogether.

Even if both the hypertension and the eclampsia are not renal in origin, the vascular changes in the kidney are inescapable. Both their etiology and rôle are equally obscure.* The intimal sclerosis found in some of the larger intrarenal arteries must be regarded as coincidental, for areas supplied by nonsclerotic arteries have equal arteriolar changes.

* Sections from this kidney were sent to Dr. Goormaghtigh, who wrote, on learning that eclampsia and mild hypertension had appeared in this patient: "It confirms me in the belief that in most cases hypertrophy of the juxtaglomerular apparatus precedes hypertension or is present in the early stages of the latter; *e.g.*, scarlet fever with glomerulonephritis, and eclamptic toxemia."

The clear appearance of the medial cells and the total absence of acidophilic fibrils suggested the possibility that these muscle cells were fixed in a state of relaxation or diminished tonus compared with fibrillar contracted muscle. That such relaxation may be due to a specific mechanism preventing tonic contractions is worthy of consideration. In this connection it may be recalled that Meigs [19] has shown that when Zenker's fluid (a form of which was employed in this case) is applied to unstriped muscle it does not cause contraction and fixes the cells at their original length.

Can pregnancy be accountable for the vascular picture? Hormonal influences come to mind and the only suggestion we can offer is indirect. Since in pregnancy the smooth muscle of the uterus, its vessels and often the lower urinary tract [20] exhibit alterations characterized by hyperplasia and hypertrophy, is it possible that in the kidney reported here we have encountered a similar effect in the renal arteries and arterioles? The description of the changes in the myometrium taken from Keiffer [21] by Reynolds [22] is worth comparing with my findings:

"The nature of the process by which hypertrophy of uterine muscle takes place during pregnancy is not generally known, although it has been most carefully studied in the guinea pig and in the human. In both species, the cells appear to imbibe water at the time the uterus begins to undergo distention with the products of conception. At the same time innumerable centripetal myofibrils envelop the sarcoplasm superficially. As progressive hydration continues the cytoplasm becomes more and more transparent while the superficial portion remains chromophilic."

The relatively clear, swollen appearance of the medial cells in my case suggests that they are possibly "hydrated," as well as relaxed.

Detailed histologic observations of vascular alterations during pregnancy have been scanty. One report, also by Keiffer,[23] may be relevant to the problems raised by my case. In a paper entitled "De l'existence d'une glande myométriale dans l'uterus humain" he has recorded histologic findings in the myometrium which include detailed studies of the uterine vessels. Noting the need for prompt fixation by quickly acting fixatives, he studied uteri obtained by cesarean section at term or almost at term, as well as uteri obtained in earlier stages of pregnancy. With photomicrographs he illustrated changes which he believed began in the intermuscular tissue and affected the vascular walls as well. Some of the latter bear a striking resemblance to the changes reported in this paper. The affected cells are mononuclear and increased in size, have finely granular cytoplasm and undergo amitotic division. In the vascular walls Keiffer observed that the smooth muscle cells also increase in size, lose their oblong shape and are finally indistinguishable from the enlarged cells of connective

tissue origin. I have confirmed this appearance in Zenker-fixed fresh uteri (Fig. 4) furnished through the courtesy of Dr. William Studdiford. Like Gérard,[24] who found similar changes in the myometrium of the mouse and rat, Keiffer found much glycogen* in the swollen cells.

Changes like these in the rabbit had first led Ancel and Boin,[25†] (1911) to propose the existence of a myometrial gland in the rabbit. Subsequently, Keiffer [21, 23] had confirmed their findings in the guinea pig and human species. But he was not certain that there was sufficient evidence to establish the existence of a myometrial "gland," and preferred the term "myometrial placenta" for the changes until evidence of secretory activity is established.

In a later review of changes occurring in the walls of the uterine arteries after parturition in the guinea pig, Prenant [26] cited several reports of the occurrence, during pregnancy in man and in other species, of giant mononuclear cells in the media of these vessels. They have been ascribed to invading decidual cells,[26] to transformed medial muscle cells,[26] or to intimal cells.[27] Further investigation of the uterine arteries and veins and similar vessels in other organs during pregnancy seems indicated. I have examined the kidneys of five patients dying at term. No similar vascular changes were encountered, but in no case was fixation accomplished in less than 1 hour after death and there may have been postmortem alteration. The lack of material from cases of fatal eclampsia has precluded search for comparable changes in that group.

If the suggestion offered is valid, and the vascular change found in one kidney was reversible, an hypothesis based on the influence of pregnancy may be offered to account for (1) the occurrence of such obvious medial hypertrophy in this patient; (2) a progressive effect on the surviving kidney with (3) consequent development of altered function, resulting (4) in hypertension and possible eclampsia, and followed (5) by the involution of such changes and recession of the hypertension with the termination of pregnancy.

The unilateral hematuria of 3 years' duration is still unexplained. There was no calculus formation, neoplasm, or chronic inflammation. The erosions in the pelvis and ureter were fresh. If similar erosions were forming and healing during the 3 years prior to nephrectomy,

* Unfortunately, the kidney reported here was not fixed in absolute alcohol, which would have been ideal for demonstrating that glycogen might account for the clear appearance.

† Gérard [24] expressed the view that the so-called myometrial gland in the rat and mouse was not the same as that described in the rabbit by Ancel and Boin. Keiffer gives no explanation for equating these structures.

they might account for the hematuria but we have no proof of their occurrence. Very little blood was found in the tubules. If related to the vascular lesions, more erythrocytes might be anticipated in Bowman's spaces or in the tubules than were found.

The peculiar appearance of the lining cells of the pelvis is also unexplained. Modern textbooks of histology make no reference to this picture in human kidneys or ureters. However, in the older literature there are observations of cellular changes like these (see Petersen [28] for a discussion of its occurrence). However, no correlation with sex, age, or physiologic activity was established. The relationship to pregnancy is being explored separately.

It is to be emphasized that the patient's blood pressure was normal in the presence of these alterations. If the medial hypertrophy commonly observed in hypertension is comparable, then this case supports the conclusion that such hypertrophy may precede hypertension rather than be a consequence of it.

Finally it may be noted that the changes in the arterioles reported here extend uniformly along the vascular channels. The so-called "Polkissen" participate equally and there is no visible difference between the end-portions of the afferent arterioles and the proximal portions. This is true for deeply placed afferent arterioles as well as for the glomerular vessels near the capsule. The swollen appearance of the cells of the "Polkissen" suggests that they are derived from the same tissue and, at least under the conditions reported here, may have abundant cytoplasm resembling smooth muscle.

Summary and Conclusions

A case is reported of chronic unilateral hematuria in a colored female, 29 years old, who was cured by nephrectomy during the fifth month of pregnancy. The removed kidney revealed unusual, massive, medial hyperplasia and hypertrophy of the arterioles. There was accompanying intimal fibromuscular hyperplasia of the interlobular branches of the renal artery. Focal fresh hemorrhagic erosions were found in the pelvis and ureter.

The medial hypertrophy could not be accounted for by any known or demonstrable injury. An hypothetical explanation is offered, based on the theory that hormonal activity during pregnancy might be responsible for changes in the arteriolar smooth muscle comparable to those found in the gravid uterus.

I am indebted to the Department of Obstetrics and Gynecology of the New York University College of Medicine for the use of the clinical data.

REFERENCES

1. Smith, H. W. Physiology of the renal circulation. *Harvey Lectures,* 1930–40, **35,** 166–222.
2. Clara, Max. Anatomie und Biologie des Blutkreislaufes in der Niere. *Arch. f. Kreislaufforsch.,* 1938, **3,** 42–94.
3. Becher, Hellmut. Über besondere Zellengruppen und das Polkissen am Vas afferens in der Niere des Menschen. *Ztschr. f. wissensch. Mikr.,* 1936, **53,** 205–214.
4. Goormaghtigh, N. Les segments neuro-myo-artériels juxta-glomérulaires du rein. *Arch. de biol., Paris,* 1932, **43,** 575–591.
5. Goormaghtigh, N. The heterogeneous structure of the arteriolar media. *J. Physiol.,* 1937, **90,** 63P–65P.
6. Goormaghtigh, N. Le cycle glandulaire de la cellule endocrine de l'artériole rénale du lapin. *Arch. de biol., Paris,* 1940, **51,** 293–311.
7. Benninghoff, A. Blutgefässe und Herz. In: von Möllendorf, Wilhelm. Handbuch der mikroskopischen Anatomie des Menschen. J. Springer, Berlin, 1930, **6,** pt. 1, 1–232.
8. Ruyter, J. H. C. Über einen merkwürdigen Abschnitt der Vasa afferentia in der Mäuseniere. *Ztschr. f. Zellforsch. u. mikr. Anat.,* 1925, **2,** 242–248.
9. Oberling, C. L'existence d'une housse neuro-musculaire au niveau des artères glomérulaires de l'homme. *Compt. rend. Acad. d. sc.,* 1927, **184,** 1200–1202.
10. Zimmermann, K. W. Über den Bau des Glomerulus der Säugerniere, Weitere Mitteilungen. *Ztschr. f. mikr.-anat. Forsch.,* 1933, **32,** 176–278.
11. Goormaghtigh, N. Existence of an endocrine gland in the media of the renal arterioles. *Proc. Soc. Exper. Biol. & Med.,* 1939, **42,** 688–689.
12. Goormaghtigh, N., and Grimson, K. S. Vascular changes in renal ischemia: cell mitosis in the media of arteries. *Proc. Soc. Exper. Biol. & Med.,* 1939, **42,** 227–228.
13. Goormaghtigh, N. Documents sur la cytologie et pathologie artériolaires; les cellules afibrillaires artériolaires dans l'ischémie rénale chez la chien. *Rev. belge sc. méd.,* 1940, **12,** 85–107.
14. Dunihue, F. M., and Candon, B. H. Histologic changes in the renal arterioles of hypertensive rabbits. *Arch. Path.,* 1940, **29,** 777–784.
15. Smith, H. W.; Goldring, William, and Chasis, Herbert. The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J. Clin. Investigation,* 1938, **17,** 263–278.
16. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia and London, 1938, p. 111.
17. Jores, Leonhard. Arterien. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1924, **2,** 701.
18. Castleman, Benjamin; Smithwick, R. H., and Palmer, R. S. Renal biopsies from hypertensive patients. (Abstract.) *Am. J. Path.,* 1941, **17,** 617–618.
19. Meigs, E. B. Striated and Smooth Muscle. In: Cowdry, E. B. Special Cytology. Paul B. Hoeber, Inc., New York, 1932, **2,** 1113.
20. Hofbauer, J. Contributions to the etiology of pyelitis in pregnancy. *Bull. Johns Hopkins Hosp.,* 1928, **42,** 118–155.
21. Keiffer, H. Des phénomènes de maturation des fibres lisses utérines, au cours de la grossesse. *Bull. Acad. roy. de méd. de Belgique,* 1928, **8,** 505–509.

22. Reynolds, S. R. Physiology of the Uterus. Paul B. Hoeber, Inc., New York, 1939, p. 120.

23. Keiffer, H. De l'existence d'une glande myométriale dans l'uterus humain. *Bull. Acad. roy. de méd. de Belgique,* 1925, **5,** 684–707.

24. Gérard, P. Sur le glande myométriale de la souris et du rat. *Compt. rend. Soc. de biol.,* 1925, **93,** 457–459.

25. Ancel, P., and Bouin, P. Sur l'existence d'une glande myométriale endocrine chez la lapine gestante. *Compt. rend. de l'Assoc. d. anat.,* 1911, pp. 97–103.

26. Prenant, A. Recherches sur les transformations de la paroi de certaines artères dans l'utérus du cobaye après parturition. *Arch. d'anat., d'histol. et d'embryol.,* 1927, **7,** 165–196.

27. Stolper, Lucius, and Herrmann, Edmund. Die Rückbildung der Arterien im puerperalen Meerschweinchenuterus. *Arch. f. mikr. Anat.,* 1904, **63,** 748–765.

28. Petersen, O. V. C. E. Ueber sekretorische Aenderungen im Epithel der ableitenden Harnwege bei einigen Säugetieren. *Anat. Anz.,* 1905, **27,** 187–199.

[*Illustrations follow*]

DESCRIPTION OF PLATE

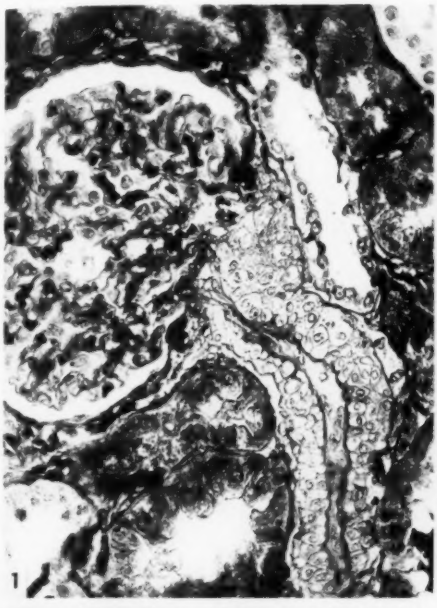
PLATE 17

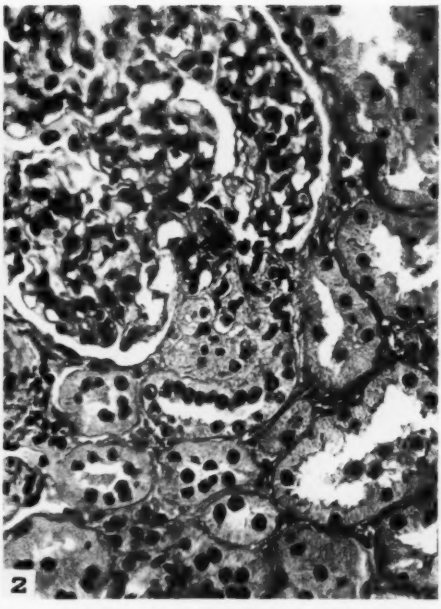
FIG. 1. Sagittal section through the terminal portion of an afferent arteriole and glomerulus showing hyperplasia and hypertrophy of the media due to afibrillar clear cells. There is an asymmetrical expansion of the juxtaglomerular portion. Azan-carmine stain. × 305.

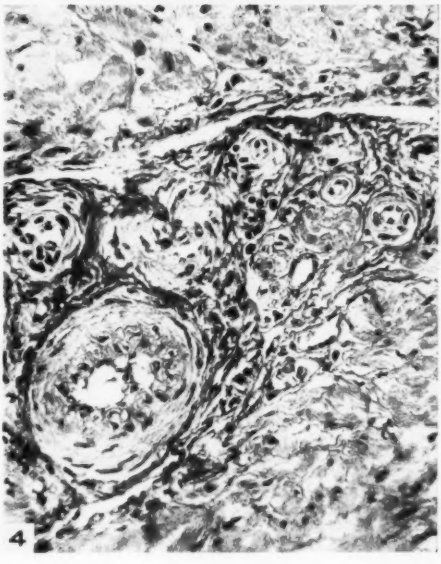
FIG. 2. Transsection through the vascular pole of a glomerulus showing the hyperplastic "Polkissen" indenting the adjacent distal tubule. The latter exhibits palisading of the nuclei in the region of contact. This formation is designated as the macula densa. Hematoxylin and picro-fuchsin stain. × 320.

FIG. 3. A view of an intralobular renal artery exhibiting fibromuscular hypertrophy of the intima and media with narrowing of the lumen. Hematoxylin and picro-fuchsin stain. × 130.

FIG. 4. A view of several uterine arterioles of different size, in cross section, showing the medial hypertrophy occurring during pregnancy (uterine tissue obtained by hysterectomy during the 14th week of pregnancy; operation performed for delayed abortion in a psychotic patient of 25 years). Hematoxylin and picro-fuchsin stain. × 245.







Graef

Medial Arteriolar Hypertrophy in Pregnancy

PATHOLOGIC CHANGES PRODUCED IN RABBITS BY A TOXIC SOMATIC ANTIGEN DERIVED FROM EBERTHELLA TYPHOSA *

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The isolation of purified antigenic materials from cultures of the Salmonella group of organisms by Boivin, Mesrobeanu and Mesrobeanu [1] and by Raistrick and Topley,[2] and the experiments of Henderson and Morgan [3] with a protein-free antigen obtained from *Eberthella typhosa* have demonstrated that substances of relative purity and high antigenic and toxic activity may be isolated from organisms of the enteric group. In recent studies I have described the preparation of a somatic antigen from *E. typhosa* cultured in a synthetic medium [4] and have presented an analysis of its toxic and immunologic properties.[5-7] The possible rôle of this material in the clinical manifestations of typhoid fever was discussed.[5] Because of its lethal effects on a variety of animals,[5] its destructive action on leukocytes [5, 6] and the inflammatory reaction that developed at the site of intradermal injection of a similar preparation employed in some earlier experiments,[8] it was decided to investigate the local and general systemic effects of this material on tissues of rabbits treated for varying periods of time by several routes of injection. The present paper describes the results of these experiments.

MATERIALS AND METHODS

Preparation of Antigen

Following the technic previously described,[4, 5] involving repeated precipitations with alcohol and resuspension in water of a material obtained from cultures of *E. typhosa* grown in a liquid medium containing only ingredients removable by dialysis,[4] an antigenic material containing the Vi antigen was prepared which possessed all of the properties of the substance studied earlier.[5]

Methods of Administration

(*a*) *Intradermal.* Rabbits were given five intracutaneous injections of the antigen and these areas of skin were excised at intervals for microscopic examination. In Table I the details of this experiment are summarized.

(*b*) *Fixation Experiments.* Following the technic of Menkin,[9] sev-

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eral rabbits received injections of the material to determine its efficacy in inducing inflammatory fixation.

(*c*) *Intravenous Injections.* Two groups of rabbits were given intravenous injections of various amounts of the material into the marginal vein of the ear at intervals of several hours. The first of these groups received injections over a much shorter period of time than did the second. Experimental details are given in Table II.

(*d*) *Intracardiac Injections.* To seven rabbits injections of antigen were administered into the left ventricle of the heart. This procedure was carried out in order to reduce, if possible, the pronounced injurious effects on the pulmonary tissue noted in the animals mentioned in (c), and to insure a maximum effect on the peripheral circulation. Table III summarizes the data.

Treatment of Tissues

Representative specimens of tissue were fixed in a 4 per cent solution of formaldehyde and in Zenker's fixative, and embedded in paraffin. Sections 7 μ in thickness were cut and stained with either hematoxylin and eosin or eosin and methylene blue.

The findings in any animals showing evidence of coccidiosis on gross or microscopic examination were excluded.

Results

Experiment 1. Local Changes Following Intradermal Injection of the Antigen

The changes induced in the tissues of the skin after various intervals of time following the injection of antigen were studied. Two adult albino rabbits were shaved over their flanks and 1 mg. of the antigen was injected intradermally into each of five areas on each animal. Segments of skin including these areas were excised at intervals of 16 to 336 hours and from them sections were prepared as indicated in Table I.

Microscopic examination of the inoculated sites removed at the end of 16 and 40 hours revealed a marked inflammatory reaction characterized by hyperemia, edema and infiltration with polymorphonuclear leukocytes and deposition of considerable amounts of fibrin. At 96 hours many of the polymorphonuclear leukocytes showed evidence of necrosis whereas the hyperemia and edema had decreased. After 168 hours the centers of the lesions had become completely necrotic and only a few degenerated polymorphonuclear cells could be distinguished. At the periphery of the lesion, fibroblastic proliferation was evident which by 336 hours had formed a fibrous capsule.

Experiment 2. The Antigenic Material as an Agent in Inducing Inflammatory Fixation

Employing the technic of Menkin,[9] two rabbits were given 5 mg. and two others 1 mg. of the material. The antigen in 1 cc. of physiologic saline solution was injected into the skin of the left foreleg; 1 cc. of physiologic saline solution was injected into the right foreleg as a control. After 18 hours, 1.5 cc. of 1 per cent trypan blue was injected into the hypodermis of each foreleg in the area previously

TABLE I
Results of Intradermal Injection of Antigen

Rabbit no.	Intradermal injection of antigen	Time after injection at which area was excised	Appearance of reaction
81 and 82	1 mg.	16 hours	Edematous, erythematous areas 2 cm. in diameter
		40 hours	Edema and erythema less intense
		4 days	Erythema absent and edema decreased; small nodules palpable in the center of the area
		7 days	Nodules have become more firm
		2 weeks	Small, firm, whitish intracutaneous nodules 0.75 cm. in diameter

treated. Each animal was sacrificed 3 hours after injection with dye and the lymphatics and lymph nodes draining the area were examined. The areas of skin into which the injections were made were excised and examined microscopically. At 18 hours, the areas injected with the antigen consisted of small (1 cm.) indurated lesions which exhibited central necrosis with surrounding zones of erythema. No significant changes were noted in the areas of the right legs injected with saline solution. On sacrificing the animal, the lymphatics of the right legs and the axillary lymph nodes were stained blue, indicating that free lymphatic drainage of the dye was present. The lymphatics and lymph nodes on the left side contained no dye. Thus inflammatory fixation depending upon lymphatic blockade had taken place.

Microscopic examination of the areas injected with the antigen showed edema and hyperemia with heavy leukocytic infiltration. The lymphatics frequently contained fibrinous thrombi and there was a network of fibrin in the interstitial spaces. Small blood vessels and capillaries were dilated and congested. In the lymph nodes the sinusoids were seen to be crowded with polymorphonuclear cells. Histologic examination of tissues from the control limbs revealed no significant abnormalities. The material under investigation therefore has the capacity to induce a marked inflammatory fixation.

Experiment 3. Pathologic Changes Induced in the Organs of Animals Following Intravascular Injections of the Antigen

These experiments may be divided into two groups, as the animals received somewhat different treatment.

The rabbits of group A were given intravenous injections over periods extending from 24 to 48 hours. At the end of various intervals, if the animals had not succumbed, they were killed by the intravenous injection of air and tissues were taken for section. The dosage and time intervals are given in Table II.

The animals in group B received intravenous and intracardiac injections over a more extended period of time. The intervals and dosage are summarized in Table III.

TABLE II

Results of Intravenous Injection of Antigen

Rabbit no.	Total amount of antigen injected	Number of injections	Number of days injected	Last dose of antigen	Manner and time of death in hours after last injection
24 and 25	0.5 mg.	1			½ Killed with intravenous air
26 and 27	0.5 mg.	1			1½ Killed with intravenous air
29	0.5 mg.	1			2 Killed with intravenous air
30 and 31	0.5 mg.	1			5 Died of injection
34	0.5 mg.	1			10 Died of injection
32 and 33	0.5 mg.	1			24 Killed with intravenous air
1	1.0 mg.	2	2	0.5 mg.	2 Died of injection
12	11.0 mg.	2	2	6.0 mg.	1½ Died of injection
4 and 48	11.0 mg.	2	2	10.0 mg.	8 Died of injection
15	1.5 mg.	4	2	0.2 mg.	3 Died of injection
17	20.0 mg.	5	1	10.0 mg.	1½ Died of injection

TABLE III

Results of Intravenous and Intracardiac Injection of Antigen

	Rabbit no.	Total amount of antigen injected	Number of injections	Number of days injected	Last dose of antigen	Manner and time of death in hours after last injection
Intravenous injections	18	6.3 mg.	8	5	5 mg.	24 Killed with intravenous air
	19	15.7 mg.	11	5	5 mg.	1 Died of injection
	20	5.0 mg.	9	4	2 mg.	24 Killed with intravenous air
	21	16.0 mg.	10	5	5 mg.	9 Killed with intravenous air
	22	15.0 mg.	16	8	3 mg.	5½ Killed with intravenous air
	23	6.0 mg.	11	8	2 mg.	24 Killed with intravenous air
	124	12.0 mg.	2	2	10 mg.	10 days Killed with intravenous air
Intracardiac injections	40	0.25 mg.	3	3	0.1 mg.	24 Killed with intravenous air
	41	0.18 mg.	3	1	0.05 mg.	24 Killed with intravenous air
	42	0.40 mg.	5	3	0.20 mg.	6 Killed with intravenous air
	43	0.50 mg.	6	3	0.20 mg.	6 Killed with intravenous air
	44	5.68 mg.	9	4	2.5 mg.	2 Killed with intravenous air
	45	7.80 mg.	6	4	5.0 mg.	3 Died of injection
	46	12.70 mg.	6	4	5.0 mg.	2 Killed with intravenous air

Gross Examination

At autopsy there were no noteworthy changes in the majority of the organs of the animals of group A. Small areas of hemorrhage were observed in the lungs of some and petechial hemorrhages were noted in the small intestine. The normal intestinal contents were frequently replaced by mucus. In group B, in addition to the above changes, some of the livers showed small, white areas scattered over the surface and in some of the animals receiving intracardiac injections there was a fibrinous pericarditis.

Microscopic Examination

Heart. Group A. In occasional animals there were scattered, small areas in which the muscle fibers stained with eosin more intensely than normal. In the more markedly altered areas, necrosis of a few muscle fibers and leukocytic infiltration were noted.

Group B. Most of the specimens from animals in this group had changes similar to those described in group A. However, in a number of the animals that received large intracardiac injections there were larger areas showing degeneration of muscle fibers. Such cells had lost their striations and coagulative necrosis of the cytoplasm was evident. In two animals, nos. 23 (Fig. 1) and 124, living for longer intervals, the degenerative changes were accompanied by a proliferation of fibroblasts and beginning cicatrization. The sections from one rabbit showed an acute fibrinous pericarditis with underlying necrosis and inflammation of the muscle tissue. This lesion was undoubtedly the result of the escape of antigen into the pericardium at the time of injection.

Lung. Group A. The alveolar capillaries and blood vessels were engorged and contained a greatly increased number of polymorphonuclear leukocytes. In some specimens hemorrhage into the alveoli and patchy atelectasis were observed; other alveoli were filled with a serous precipitate. These changes were most marked in the animals given the larger dosage.

Group B. Areas of atelectasis and the accumulations of polymorphonuclear leukocytes in alveolar capillaries were also seen in this group. Some alveoli contained masses of polymorphonuclear leukocytes as well as serous precipitate. Thrombosis of small and medium-sized blood vessels was present in several animals (Fig. 2). Occasionally there was evidence of early stages of organization of the thrombi (Fig. 3). In two rabbits, nos. 22 and 24, focal areas of inflammatory consolidation were noted. These contained collections of mononuclear

leukocytes. The alterations in the lungs were less marked in the animals in which the antigen was injected into the left ventricle.

Liver. Group A. In the animals given the smaller dosage, scattered, isolated liver cells or small groups of cells exhibited hyalinization of the cytoplasm and pyknosis of the nuclei. Polymorphonuclear leukocytes had invaded or collected around the more extensively damaged cells. Following larger doses of antigen, definite areas of focal necrosis of hepatic cells were observed and many cells had entirely lost their structure. The blood vessels were engorged with erythrocytes.

Group B. The above-mentioned changes were better defined in the livers of this series of animals (Fig. 4). In occasional lesions, mononuclear phagocytes were present in the sinusoids about the areas of focal necrosis (Fig. 5). In animals nos. 44 and 45, which died soon after large, intracardiac injections of the antigen, extensive areas of necrosis of liver cells with few polymorphonuclear leukocytes in the sinusoids were observed. In addition to these changes, there were numerous occluding or mural thrombi in the central hepatic and larger veins (Fig. 6). These thrombi consisted of masses of an eosinophilic, hyaline material in which degenerating polymorphonuclear leukocytes were embedded and which were often surrounded by a network of fibrin.

Intestine. No constant pathologic changes were noted in the small intestines of either group. However, in some of the animals receiving large amounts of the antigen, there were indications of cellular necrosis and phagocytosis in the centers of some of the lymphoid follicles.

Spleen. The changes observed in the spleens of the animals in both groups were variable. Frequently the sinusoids, in rabbits killed after the longer intervals following the last injection, contained excessive accumulations of polymorphonuclear leukocytes. Occasionally thrombi were also observed in the vascular spaces. Phagocytosis of cells, cell fragments and an eosinophilic material by the mononuclear cells lining the sinusoids was apparent. In some of the specimens this phagocytic activity appeared considerably increased. Increased mitotic activity was also evident in some instances in the cells of the lymphoid follicles.

Adrenal. In both groups A and B, microscopic examination revealed varying degrees of degenerative changes in the cortical cells. In many there was evidence of injury or necrosis of isolated cortical cells. In the glands obtained from animals nos. 19 and 124 occasional small groups of cells had become necrotic and were invaded by polymorphonuclear leukocytes.

Kidney. The changes in these organs from both groups A and B

consisted mainly of congestion and hyperemia of the blood vessels. In rabbits nos. 19 and 20, thrombosis of the capillaries of the glomerular tufts and acute, tubular degeneration were noted.

Bone Marrow. Group A. In the animals dying within 1 to 3 hours after the last injection, the marrow was observed to contain relatively few adult polymorphonuclear cells. With longer periods following the final injection, the bone marrow contained large numbers of young polymorphonuclear leukocytes and myelocytes. In some animals there was an extravasation of blood into the marrow spaces.

Group B. The sections of the bone marrow were characterized by a marked hyperplasia of cells of the granulocytic series and alterations of the megakaryocytes marked by pyknosis of the nuclei and occasional invasion of the cell by polymorphonuclear leukocytes. Areas of necrosis and mononuclear cell infiltration were seen in the specimens from a few of the animals. Phagocytic cells were seen in many cases to have ingested fragments of the necrotic cells.

These changes, although variable, were more pronounced in the series of animals that received the antigen by the intracardiac route. Widespread necrosis of cells with or without hemorrhagic extravasation was most marked in the animals receiving the larger amounts of antigen shortly before death in whose bone marrow only a few mature leukocytes remained. There was hyperplasia of immature cells of the granulocytic series with increased mitotic activity which led them to overshadow the erythroblastic cells. Thrombi similar to those described in the liver and spleen were occasionally observed in the vascular sinuses of the marrow.

Discussion

These experiments are of interest in that they indicate that the somatic antigen of *E. typhosa,* which is released on disintegration of the bacterial cell, has toxic effects on local injection and widespread destructive action when it is carried by the blood stream into various tissues. As previously reported,[5] the animals give evidence of its toxic action on injection as indicated by labored respiration. Many also have a profuse diarrhea and their temperatures rise from 101° or 103° F. to levels as high as 105° to 107° F.

The congestion and hemorrhages in various organs as noted at autopsy have been described by Arima [10] in animals treated with a toxic solution derived from cultures of the typhoid bacillus. Similar observations and necrosis of liver cells in rabbits were recorded by Spanedda [11] and Dennis [12] who used toxic substances prepared from *E. typhosa* by the method of Boivin.[1]

The toxic action of the antigen on liver cells, lymphoid tissue, cardiac muscle and polymorphonuclear leukocytes is most prominent. From the evidence presented, it may be concluded that the destructive action of the antigen on leukocytes is not exerted solely against the polymorphonuclear leukocytes in the circulating blood as observed in previous experiments using rabbits [5] and human beings,[13] but that the substance reaching the bone marrow via the blood stream also destroys the cells *in situ*, producing areas of necrosis in some instances. Likewise the material has been demonstrated to destroy hepatic cells with a production of focal necroses. The lesions in the liver and bone marrow may, by their location, suggest some relationship to the focal necroses found in the bone marrow and liver of patients dying of typhoid infection as described by Mallory.[14] It is perhaps noteworthy that these lesions in the human material occur without the presence of demonstrable typhoid bacilli in the sections, as pointed out by Mallory [14] and Goodpasture.[15] Goodpasture has postulated that these focal necroses indicate the action of an endotoxin in the circulating blood derived from phagocytosed and lysed bacilli. Thus the powerful tissue toxicity of the antigen used in these experiments may fulfill this rôle since its toxicity for these tissues has been demonstrated and it is released on the disintegration of typhoid organisms.

The occurrence of thrombi in the vessels of the lung, liver, spleen and in the sinusoids of the liver, spleen and bone marrow gives evidence that this material injures vascular endothelium leading to thrombosis. This action may possibly be related to the mechanism responsible for the widespread thrombosis of blood vessels in the tissues of patients dying of typhoid fever,[14] though the collections of large mononuclear cells within the vessels and beneath the endothelium as described by Mallory [14] were not reproduced in these experimental animals.

Thus, although the lesions in the tissues of these rabbits injected with the toxic antigen derived from *E. typhosa* have in many instances shown no striking cytologic similarity to the lesions observed in tissues of fatal cases of typhoid fever in man, the production of lesions in the liver, spleen, bone marrow and blood vessels indicates that this potent tissue toxin may play a rôle in the production of changes observed in the tissues of patients dying of typhoid fever.

Summary

1. The toxic somatic antigen isolated from *E. typhosa* produces a marked inflammatory reaction when injected into the skin of a rabbit that leads to fixation *in situ* of an injected colloidal dye. The

resultant changes proceed to eventual necrosis and walling off of the area by fibrous tissue.

2. The antigen administered by intravascular routes causes widespread injury to capillary and blood vessel walls with subsequent thrombosis, and leads to the necrosis of cells of the liver, heart muscle, adrenal glands and bone marrow. The kidneys and the lymphoid tissue of the spleen and small intestine may also be injured. The injurious action of the antigen is followed by a hyperplasia of the bone marrow chiefly involving the granulocytic series of cells.

I wish to acknowledge with gratitude the advice and constructive criticism of Dr. G. A. Bennett of the Department of Pathology of the Harvard Medical School. The experiments on inflammatory fixation were carried out by Dr. Valy Menkin of the Department of Pathology. The author wishes to thank Dr. J. A. Horneff for assistance in the preparation of the photomicrographs.

REFERENCES

1. Boivin, André; Mesrobeanu, Ion, and Mesrobeanu, Lydia. Technique pour la préparation des polysaccharides microbiens spécifiques. *Compt. rend. Soc. de biol.*, 1933, **113**, 490–492.
2. Raistrick, H., and Topley, W. W. C. Immunizing fractions isolated from *Bact. aertrycke*. *Brit. J. Exper. Path.*, 1934, **15**, 113–130.
3. Henderson, D. W., and Morgan, W. T. J. The isolation of antigenic substances from strains of *Bact. typhosum*. *Brit. J. Exper. Path.*, 1938, **19**, 82–94.
4. Morgan, H. R. Preparation of antigenic material inducing leukopenia from *Eberthella typhosa* cultured in a synthetic medium. *Proc. Soc. Exper. Biol. & Med.*, 1940, **43**, 529–532.
5. Morgan, H. R. Immunologic properties of an antigenic material isolated from *Eberthella typhosa*. *J. Immunol.*, 1941, **41**, 161–180.
6. Morgan, H. R., and Upham, H. C. Effect of antigenic material from *Eberthella typhosa* upon migration of guinea pig leucocytes. *Proc. Soc. Exper. Biol. & Med.*, 1941, **48**, 114–115.
7. Cundiff, R. J., and Morgan, H. R. The inhibition of the bactericidal power of human and animal sera by antigenic substances obtained from organisms of the typhoid-Salmonella group. *J. Immunol.*, 1941, **42**, 361–367.
8. Morgan, H. R., and Beckwith, T. D. Immunological relationships of polysaccharides of mucoid organisms of the typhoid-Salmonella group. *J. Bact.*, 1939, **37**, 389–399.
9. Menkin, Valy. Studies on inflammation. I. Fixation of vital dyes in inflamed areas. *J. Exper. Med.*, 1929, **50**, 171–180.
10. Arima, R. Ueber die Typhustoxine und ihre pathogene Wirkung. *Zentralbl. f. Bakt.*, 1 Abt. Orig., 1912, **65**, 424–436.
11. Spanedda, Antonio. Sul polisaccaride del B. tifico. VI. Ancora sull'azione tossica. *Boll. Soc. ital. biol. sper.*, 1937, **12**, 143–144.
12. Dennis, E. W. Toxicity of acid-soluble typhoid toxin for laboratory animals. *Proc. Soc. Exper. Biol. & Med.*, 1939, **42**, 553–554.
13. Morgan, H. R. Unpublished experiments.
14. Mallory, F. B. A histological study of typhoid fever. *J. Exper. Med.*, 1898, **3**, 611–638.
15. Goodpasture, E. W. Concerning the pathogenesis of typhoid fever. *Am. J. Path.*, 1937, **13**, 175–185.

DESCRIPTION OF PLATE

PLATE 18

FIG. 1. Cardiac muscle from rabbit no. 23. Degeneration of cardiac muscle fibers with beginning cicatrization is present. Hematoxylin and eosin stain. × 200.

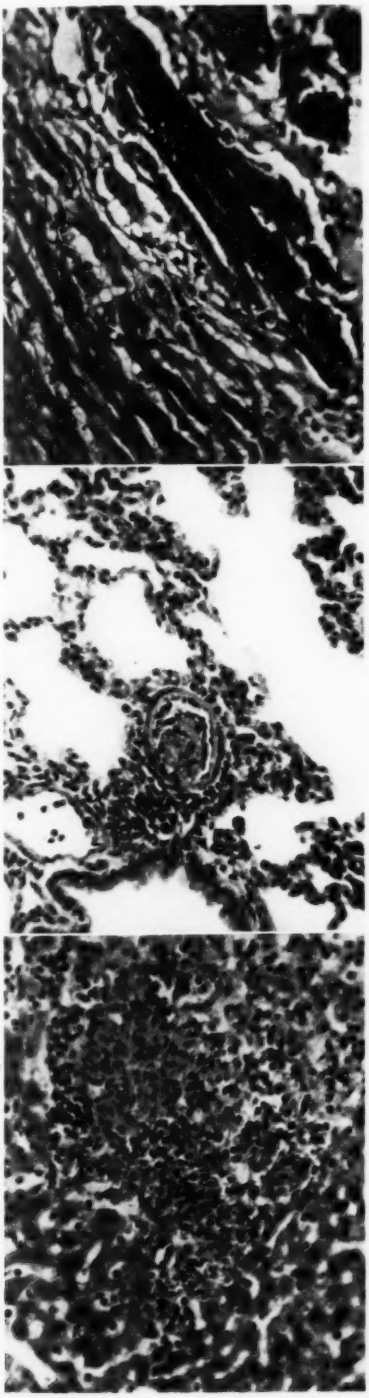
FIG. 2. Lung of rabbit no. 45. Thrombosis of a medium-sized vein and large numbers of polymorphonuclear leukocytes within the alveolar walls are notable. Eosin and methylene blue stain. × 200.

FIG. 3. Lung of rabbit no. 18. The thrombus in this small blood vessel is observed in an early stage of organization with a layer of endothelium extending over its surface. Hematoxylin and eosin stain. × 200.

FIG. 4. Liver of rabbit no. 20. Liver cells in this area have undergone degenerative changes and invasion by polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 200.

FIG. 5. Liver of rabbit no. 18. In this area of necrosis there is complete loss of the liver parenchyma and infiltration by mononuclear cells. Hematoxylin and eosin stain. × 200.

FIG. 6. Liver of rabbit no. 44. A hyaline thrombus lies within the lumen of this hepatic vessel. Eosin and methylene blue stain. × 200.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EXPERIMENTAL NECROTIZING ARTERITIS IN DOGS

III. Bilateral Nephrectomy as Effective as Heavy Metal Injury in its Production *

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In a recent publication I [1] described acute necrotizing arterial lesions which appeared unexpectedly during the course of experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level. Five dogs, maintained on a standard low protein diet, were made hyperproteinemic by repeated daily injections of plasma obtained from healthy donor dogs. Each then received a single subcutaneous injection of uranium nitrate; 5.0 mg. per Kg. Four of these showed acute necrotizing arterial lesions when they died 8 to 17 days later and the fifth dog showed healed lesions in the pulmonary artery when it was sacrificed 11 months later. Four additional dogs, maintained on the same standard diet, were made hypoproteinemic by repeated plasmapheresis, then injected subcutaneously with uranium nitrate. The two dogs which received 3.0 and 5.0 mg. per Kg. showed similar arterial lesions when they died 15 days after injection of the heavy metal, while the two dogs which received 2.0 and 2.5 mg. per Kg. failed to develop any arterial lesions.

These arterial lesions affected principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries), but were also found in other arteries (mesenteric, femoral, subclavian). They were consistently absent from the vessels of organs other than the heart and lungs.

Essentially, the lesion is an acute necrotizing arteritis apparently starting, and usually most marked, in the intima but not infrequently involving all three coats. In a few instances the lesion was situated primarily in the adventitia. Edema, together with swelling and fragmentation of collagen, seemed to be the initial change. In rapid succession, necrosis of collagen, outpouring of fibrin, massive polymorphonuclear reaction and disintegration of elastic tissue and the other elements in the arterial wall ensued. The process appeared to start in the connective tissue beneath intact endothelium, but in the advanced lesions ulceration with thrombosis was observed. Sometimes calcium was deposited in the necrotic lesions. Some of the lesions

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resembled those of periarteritis nodosa and rheumatic arteritis, but no claim of identity was made.

In a more recent article,[2] identical lesions in the same locations have been reported following the use of mercuric chloride instead of uranium nitrate. Two dogs, maintained on the same standard diet, were made hyperproteinemic by repeated daily injections of plasma obtained from healthy donor dogs. Each then received a single intravenous injection of mercuric chloride; 3.0 mg. per Kg. Both dogs showed acute necrotizing arterial lesions when they died 6 and 7 days after injection of the heavy metal. One of these dogs had a saccular aneurysm about 8 mm. in diameter situated on the anterior surface of the innominate artery immediately at its point of origin from the arch of the aorta.

Thus these experimental necrotizing arterial lesions have been produced with regularity by paying attention to three factors:

1. Standard low protein diet.

2. Plasma alteration (usually repeated intravenous injections of plasma obtained from healthy donor dogs).

3. Heavy metal injury (both uranium nitrate [1] and mercuric chloride [2]). Control observations indicated that the lesions were related to the experimental procedure and were not due to accidental or coincidental infection. Although some conjectures about the pathogenesis of the lesions have been made,[1] no satisfactory explanation for them has been advanced.

The present report attempts to elucidate one of the three factors, namely, heavy metal injury. Since both uranium nitrate and mercuric chloride produce renal injury with death in "uremia," the question naturally arose whether the arterial lesions are related to the heavy metal as such or whether they are due to the renal insufficiency which follows the injection of the heavy metal. To bring evidence to bear on this point it was decided to substitute bilateral nephrectomy for heavy metal injury. At the outset it was realized that the dogs might not live long enough to decide the question, but since the lesions had been observed as early as 6 days after mercuric chloride and since survivals of longer intervals after bilateral nephrectomy had been reported,[3] the attempt was made and has been rewarded by finding less marked lesions, presumably in the early stages of their development.

Methods

The methods have been described in detail in previous publications.[1, 2] The three young, adult, female dogs used in the present studies were maintained on the standard diet which consisted of:

calves' liver (raw wet weight), 32 parts; cane sugar, 25 parts; cornstarch, 25 parts; butter, 12 parts; cod liver oil, 6 parts. Enough tomato juice was added to make a paste, of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per Kg. per day. One gm. of the McCollum-Simmonds salt mixture [4] and 5 gm. of kaolin were thoroughly mixed with each day's diet.

The plasma injections, repeated six times per week for 3 to 4 weeks, averaged 102 cc. This amounted to one-fourth to one-third of the total quantity of circulating blood plasma in the recipient, and during the course of 3 to 4 weeks the total citrated plasma injected amounted roughly to five to six times the total volume of circulating blood plasma of the recipient. Each injection contained about 2.5 cc. of a saturated aqueous solution of trisodium citrate.

Nembutal anesthesia (40 mg. per Kg. in about 10 cc. of sterile distilled water injected intraperitoneally) was employed for the nephrectomy operations. In dog no. 40-52 the left kidney was removed 4 months before starting the plasma injections. The right kidney of dog no. 40-52 and both kidneys of the other two dogs were removed on the day following the last injection of plasma. The incision was paracostal in the anterior axillary line and the chief precaution taken in removal of the kidneys was the careful stripping of the perirenal tissues so as to expose the pedicle (artery, vein and ureter). This was tied with number 9 white silk thread that had been doubled twice to form four strands. No dressing was necessary. Sterile technic was observed throughout.

Necropsy was performed promptly after death of the dogs on the fifth or sixth day after removal of the kidneys. Blocks from all of the organs and from most of the tissues were fixed in a 4 per cent solution of formaldehyde and in Zenker's solution. Routine sections, made from the Zenker-fixed material embedded in paraffin and cut at 7 μ, were stained with hematoxylin and eosin. Special stains used in selected cases included: Verhoeff's stain for elastic tissue, sometimes combined with van Gieson's picro-acid fuchsin solution; von Kossa's silver nitrate method for calcium, scarlet red stain for fats; phloxine-methylene blue and Gram's stain for bacteria; and Levaditi's stain for spirochetes.

Experimental Observations

Table I gives in summary the experimental data and in Table II the size and anatomical distribution of the lesions are indicated.

Typical lesions are illustrated in Figures 1, 2, 4 and 7. Qualitatively the lesions were identical with those previously reported following the

use of uranium nitrate [1] and mercuric chloride.[2] Leukocytic infiltration with nuclear fragmentation is definite in all of these figures. While most of the leukocytes are most accurately described as "cells with distorted nuclei," some of them are recognizable as polymorphonuclear leukocytes. A rare mononuclear cell with faintly eosinophilic cytoplasm—presumably an eosinophilic myelocyte—has been observed,

TABLE I

*Summary of Experimental Data on Dogs with Bilateral Nephrectomy**

Dog number	Number of injections†	Total amount of plasma injected	Plasma protein concentration		Body weight‡	Height of non-protein nitrogen	Survival interval¶
			Before injection	After injection			
		(*cc.*)	(*gm. per 100 cc.*)	(*gm. per 100 cc.*)	(*Kg.*)	(*mg. per 100 cc.*)	(*days*)
40–52	24	2515	7.7	10.2	7.3	710	5
40–77	24	2515	7.1	9.3	5.2	295§	6
42–1	19	1825	5.9	8.3	5.6	538	6

* All dogs maintained on basal diet throughout entire period.
† The total period in which these injections were made was 3 to 4 weeks.
‡ Body weight at end of period of plasma injections.
§ This determination was made 2 days before this dog died.
¶ Interval between bilateral nephrectomy and death.

TABLE II

*Size and Distribution of Arterial Lesions**

Dog number	Ascending aorta	Left auricle	Pulmonary artery	Other arteries
40–52	+†	+		
40–77	+	+†	+	Myocardial arteriole, +†
42–1	++†	++	+	Innominate, +; arterioles in myocardium, +† and in submucosa of stomach, +.

* The lesions have been graded as follows: ++ = gross lesion less than 1 cm. in maximum diameter; + = lesion discovered in microscopic section.
† Microscopic illustration.

but eosinophils were not a conspicuous part of the reaction. Edema with swelling and early fragmentation of collagen can be seen about the margins of some of the lesions (Figs. 2 and 4). Verhoeff's stains showed definite fragmentation of elastic tissue in some of the lesions (Fig. 5). No calcification nor thrombosis was seen in these early lesions. Frozen sections stained for fat have been negative.

Quantitatively the lesions were definitely smaller than those that have previously been reported.[1, 2] In all probability they would have been overlooked in a routine necropsy. Careful examination of the areas in which the lesions were known to occur most frequently (pulmonary artery, endocardium of the left auricle, ascending limb of

the arch of the aorta including the mouth of the innominate artery) revealed a grossly positive result in only one dog. In the other two dogs there was questionable roughening of the intimal or endocardial surface in one or more foci that proved positive on histological examination.

As in previous experiments no organisms have been cultured from the blood during life or from the lesions at the time of necropsy. Nor have any organisms been demonstrated in or about the lesions with methylene blue, Gram's or Levaditi's stains. Evidence that sodium citrate, the anticoagulant injected with the donor's plasma, was not responsible for the development of the lesions has recently been published.[5] Thus all control data continue to indicate that the lesions are related to the experimental procedure and are not due to accidental or coincidental infection.

Discussion

From the results reported above, I believe the statement is justified that bilateral nephrectomy is as effective as heavy metal injury in the production of these necrotizing arterial lesions. The negative gross results in two of the dogs are probably related to the fact that the animals did not survive the operation for a sufficient period of time. In the positive animals the lesions are similar in all qualitative respects. Thus the question of heavy metal as such has been eliminated and the essential factor appears to be renal insufficiency.

The similarity of some of the lesions to those of periarteritis nodosa is evident from Figure 6 and has been commented on previously.[1] In this connection it is of interest that many human cases of periarteritis nodosa are preceded by diffuse glomerulonephritis.[6] A survey of the 20 cases of periarteritis nodosa in the files of the Department of Pathology of Columbia University substantiates this view. In 12 of the 20 cases there was definite evidence of nitrogen retention and in the majority it was evident that the renal insufficiency antedated the arterial changes.*

The way in which renal insufficiency operates in the production of arterial lesions has interested clinicians since the time of Hippocrates and especially since the time of Richard Bright. Considerable impetus has recently been added to the problem by the work of Goldblatt in which he has shown that renal ischemia of moderate degree leads to hypertension, while renal ischemia severe enough to produce insufficiency with nitrogen retention leads to hypertension and arteriolar necrosis.[7] Neither the pressor substance nor the necrotizing factor

* The author is indebted to Dr. J. W. Jobling for access to these files.

has been isolated but the latter appears to be definitely correlated with renal insufficiency. Winternitz and Katzenstein [8] have reached the same conclusion regarding the rôle of renal insufficiency in the necrotizing arterial lesions which they produced in dogs by subtotal ureteral obstruction.

Concerning the manner in which renal insufficiency might operate in the pathogenesis of these arterial lesions, three possibilities come to mind:

1. Toxic factor—failure to eliminate something toxic to the arterial wall.

2. Nutritive factor—removal of something necessary for continued arterial integrity.

3. Imbalance—indirectly upsetting a balance necessary for arterial integrity. No evidence is available to substantiate any of these general possibilities.

The rôle of one of the other two factors—plasma alteration—is discussed in the following paper.

Summary

1. Acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries) have been produced with regularity in dogs by combining three factors:

a. Maintenance on a standard low protein diet.

b. Plasma alteration (usually repeated daily injections of plasma obtained from healthy donor dogs).

c. Heavy metal injury—both uranium nitrate [1] and mercuric chloride.[2]

2. In the present report it has been shown that bilateral nephrectomy can be substituted for heavy metal injury, thus indicating that renal injury rather than heavy metal as such is the essential factor.

REFERENCES

1. Holman, R. L. Acute necrotizing arteritis, aortitis, and auriculitis following uranium nitrate injury in dogs with altered plasma proteins. *Am. J. Path.*, 1941, **17**, 359–375.

2. Holman, R. L., and Hewitt, W. C. Experimental necrotizing arteritis. II. Mercuric chloride as effective as uranium nitrate in its production. *Proc. Soc. Exper. Biol. & Med.*, 1942, **49**, 58–62.

3. Winternitz, M. C.; Mylon, E.; Waters, L. L., and Katzenstein, R. Studies on the relation of the kidney to cardiovascular disease. I. *Yale J. Biol. & Med.*, 1940, **12**, 623–679.

4. McCollum, E. V., and Simmonds, N. A study of the dietary essential, water-soluble B, in relation to its solubility and stability towards reagents. *J. Biol. Chem.*, 1918, **33**, 55–89.

5. Donnelly, G. L., and Holman, R. L. The stimulating influence of sodium citrate on cellular regeneration and repair in the kidney injured by uranium nitrate. *J. Pharmacol. & Exper. Therap.*, 1942, **75**, 11–17.
6. Klemperer, Paul. Discussion of a case of generalized necrosing arteritis presented before the New York Pathological Society by C. T. Olcott. *Arch. Path.*, 1932, **13**, 354–355.
7. Goldblatt, Harry. Experimental hypertension induced by renal ischemia. *Harvey Lectures*, 1937–38, **33**, 237–275.
8. Winternitz, M. C., and Katzenstein, R. Studies on the relation of the kidney to cardiovascular disease. II. *Yale J. Biol. & Med.*, 1940, **13**, 15–38.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 19

Necrotizing arterial lesions in dogs. All sections were taken from dog no. 42-1 and were stained with hematoxylin and eosin.

FIG. 1. Acute necrotizing arteritis at point of origin of innominate artery from aorta. × 85.

FIG. 2. (From area indicated in Fig. 1.) × 380.

FIG. 3. Right ventricle. Necrotizing arteriolitis. × 380.

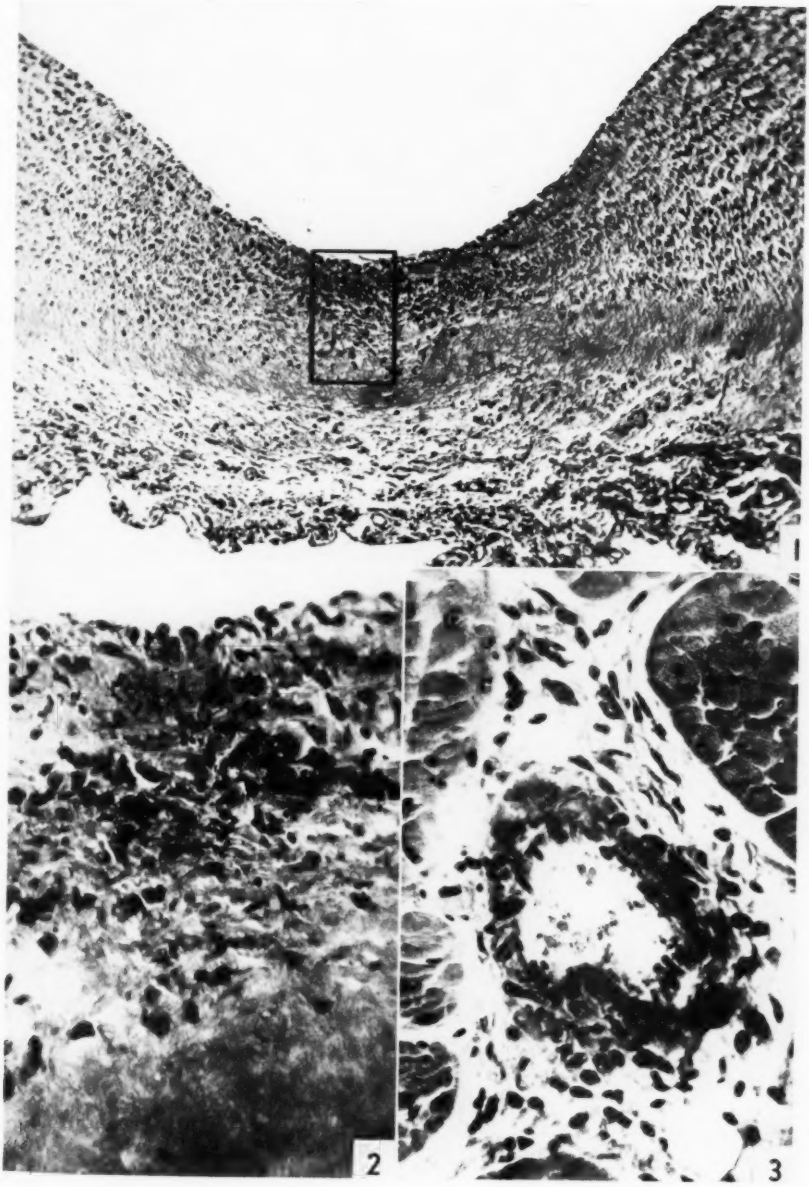
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PLATE 20

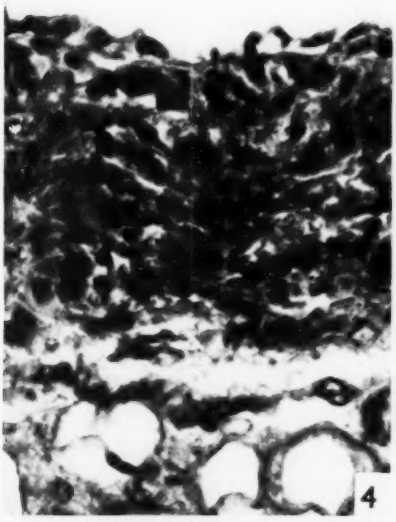
Necrotizing arterial lesions in dogs. All sections, except that used for Figure 5, were stained with hematoxylin and eosin.

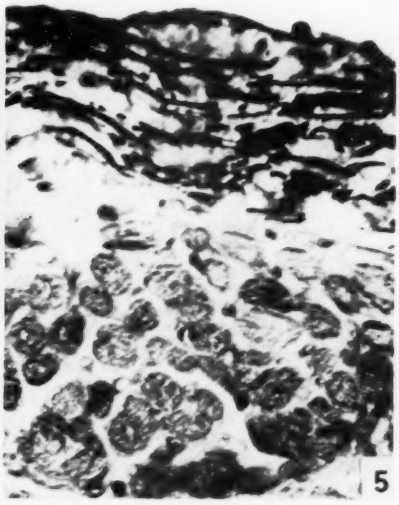
FIG. 4. Dog no. 40-77. Acute necrotizing left auriculitis. × 520.

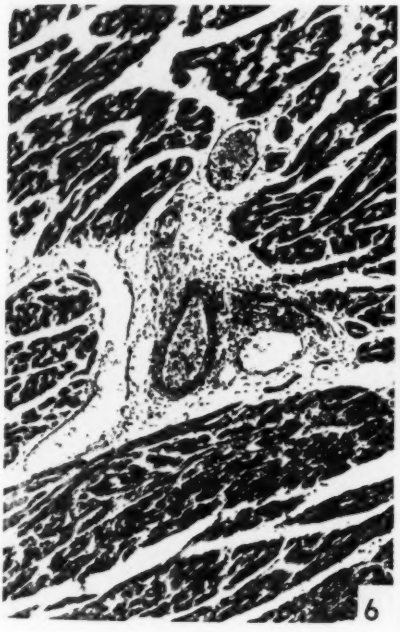
FIG. 5. Dog no. 40-77. Acute necrotizing left auriculitis. Swelling and fragmentation of elastic tissue. Verhoeff's elastic tissue stain. × 350.

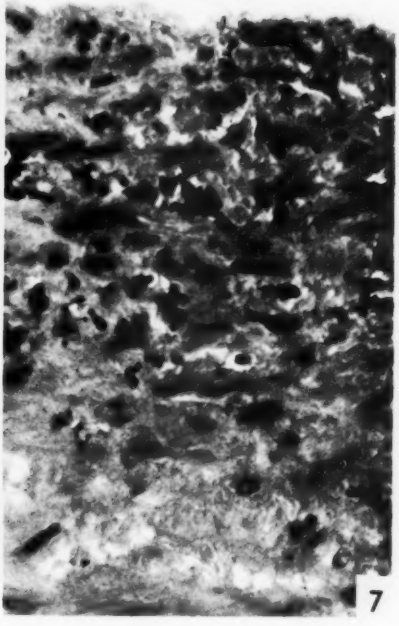
FIG. 6. Dog no. 40-77. Left ventricle. Necrotizing arteriolitis simulating periarteritis nodosa. × 95.

FIG. 7. Dog. no. 40-52. Acute necrotizing aortitis. × 520.









Holman Experimental Necrotizing Arteritis. III.

EXPERIMENTAL NECROTIZING ARTERITIS IN DOGS

IV. Alteration of the Blood Plasma Proteins Not Essential *

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In previous publications [1-3] evidence has been presented indicating that acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries) can be produced with regularity in dogs by controlling three factors:

1. Standard low protein diet.
2. Plasma alteration (usually repeated intravenous injections of citrated plasma obtained from healthy donor dogs).
3. Renal insufficiency produced by toxic doses of uranium nitrate,[1] or mercuric chloride,[2] or by bilateral nephrectomy.[3]

All of the control data have indicated that the lesions are related to the experimental procedure and are not due to accidental or coincidental infection.

In this paper data are presented which eliminate from an essential rôle one of the most puzzling of these three factors, namely, plasma alteration. If these observations are confirmed, the problem is reduced to two factors: (1) a dietary factor and (2) a factor associated with renal insufficiency.

During the course of experiments by Donnelly and Holman,[4] designed originally to determine whether sodium citrate (the anticoagulant used in obtaining plasma from the donor dogs) played any pathogenic rôle in these arterial lesions, 3 of 66 dogs receiving "citrate and saline" instead of "citrate and plasma" showed typical necrotizing arterial lesions when they died 10 to 14 days after a lethal dose of uranium nitrate or mercuric chloride. These were the first examples of necrotizing arterial lesions that had been encountered in a dog not maintained on the standard diet and not subjected to "plasma alteration."

About this same time Dr. M. C. Winternitz delivered a lecture at Duke University Medical School in which he presented illustrations of "hemorrhagic" and "necrotic" lesions in various organs and tissues of dogs that had been subjected to subtotal ureteral obstruction. Some of these lesions involved arteries and arterioles and resembled the

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necrotizing lesions that we had been obtaining. No mention was made of the diet on which his animals were maintained, and no "plasma alteration" was involved in his experiments. Some of the data which he presented at that time have since been published.[5, 6]

These observations, plus the disconcerting fact that lesions had been observed in hypoproteinemic as well as hyperproteinemic dogs,[1] prompted the following experiments using *prolonged maintenance on the standard diet* before producing renal injury.

TABLE I

Summary of Experiments without "Plasma Alteration"

Dog number	Period on standard diet* (*months*)	Type of renal injury	Maximum nonprotein nitrogen (*mg. per 100 cc.*)	Survival interval† (*days*)
40–56	5	Previous left nephrectomy, right nephrectomy	20	0
40–83	5	Bilateral nephrectomy	348	3
40–85	5½	Bilateral nephrectomy	308	4
40–84	5	Bilateral nephrectomy	244‡	5
40–79	4	Previous left nephrectomy, uranium nitrate, 5.0 mg./Kg.	544†	7
40–68	3	Uranium nitrate, 5.0 mg./Kg.	162§	35 (sacrificed)

* Period before renal injury was produced.
† Interval between renal injury and death.
‡ Last sample obtained on day before death.
§ Seven days after renal injury. Terminal nonprotein nitrogen, 44 mg./100 cc.

METHODS

The methods were the same as those outlined in the preceding paper.

EXPERIMENTAL OBSERVATIONS

Table I gives in summary the experimental data on the six dogs maintained on the standard diet for 3 to 5½ months before being subjected to interference with renal function. This consisted of bilateral nephrectomy in four of the dogs and of uranium nitrate injury (5.0 mg. per Kg. injected subcutaneously) in the other two dogs. One of the latter, dog no. 40-79, and one of the former, dog no. 40-56, had had the left kidney removed several months before commencement of the experiments with standard diet feeding. Four of the dogs died in "uremia" 3 to 7 days after the renal injury; one, dog no. 40-68, survived the renal injury and was sacrificed 35 days later; the other died of an operative accident. This last dog, no. 40-56, served as a control on diet alone. In this connection many of the dogs used in the studies on plasma protein regeneration at the University of Rochester have been maintained on the same standard diet for periods

of over 1 year and have not shown necrotizing arterial lesions.[7] The only feature of note in the kidneys excised after several months on the standard diet was the presence of considerable fat, readily stainable by scarlet red, in the loops of Henle.

The size and distribution of the arterial lesions are indicated in Table II. No gross lesions were seen in dogs nos. 40-83 and 40-85. In dog no. 40-85 early lesions were present in the microscopical sections while in dog no. 40-83, which survived bilateral nephrectomy

TABLE II

*Size and Distribution of Arterial Lesions**

Dog number	Ascending aorta	Left auricle	Pulmonary artery	Other arteries, and remarks
40–56				Control dog; no arterial lesions
40–83		±	±	
40–85	+	+	+	
40–84	++	++†	++†	Innominate ++† (aneurysmal bulge)
40–79	++	+++	+++	Innominate +++ (aneurysm); common carotid ++†; subclavian ++; internal mammary ++; femoral ++; mesenteric +
40–68	++	+++	+++†	Artery in lung+†; all lesions healing

* The lesions have been graded as follows: +++ = gross lesions over 1 cm. in maximum diameter; ++ = gross lesions less than 1 cm. in maximum diameter; + = lesions discovered in microscopical sections; ± = equivocal lesions.

†Microscopical illustration.

only 3 days, suggestive changes (edema, swelling of collagen, early pyknosis of nuclei and questionable leukocytic infiltration) were seen on histological examination, but no definite necrosis was present. In the other three dogs (nos. 40-84, 40-79, and 40-68) typical gross and microscopical lesions were found in the usual locations (Figs. 1-7; see preceding paper for description of lesions).

It is noteworthy that the findings in the nephrectomized dogs were directly proportional to the length of time the animals survived the operation. One (dog no. 40-56) died of an operative accident; one (dog no. 40-83, 3 days) was grossly negative and showed only suggestive changes on histological study; one (dog no. 40-85, 4 days) was grossly negative but showed definite microscopical lesions; and one (dog no. 40-84, 5 days) showed typical gross and microscopical lesions including an aneurysmal bulge on the anterior surface of the innominate artery at its point of origin from the arch of the aorta. Previously, gross lesions have not been observed earlier than 6 days after the production of renal insufficiency.

Two features of the lesions in dog no. 40-79 deserve special comment. First, there was a saccular aneurysm 9 mm. in diameter and partially filled with laminated thrombus on the anterior surface of

the innominate artery at its point of origin from the aorta. This is the second such aneurysm that has been found and both occurred at the same site (see dog no. 40-80[2] for the other). The second feature of interest is that the lesions differ to some extent from the ones usually seen. Instead of massive leukocytic infiltration and necrosis with partial liquefaction of the elements of the arterial wall, the lesion was chiefly a change in the elastic tissue. Grossly the affected vessels were crumpled and inelastic. Microscopically, instead of being undulating the internal elastic membrane was in a straight line (Fig. 5). Necrosis was obvious from the loss of nuclei and the more intense eosin staining of the smooth muscle cells, but leukocytic infiltration was minimal. These lesions resembled those previously described and illustrated in dog no. 38-24,[1] except that calcium deposition was much more conspicuous in dog no. 38-24. Possibly this was related to the time factor; dog no. 40-79 lived only 7 days after the renal injury while dog no. 38-24 survived 15 days. Renal injury in both was produced by uranium nitrate.

All of the lesions in dog no. 40-68 were healing or healed. This dog survived the acute phase of the experiment and was sacrificed with ether 35 days after the administration of uranium nitrate. This is the second dog that has shown healed lesions (compare dog no. 39-28 that was sacrificed 11 months after the administration of 5.0 mg. of uranium nitrate per Kg.[1]). In dog no. 39-28 there was a complication—the presence of five adult female heart worms, *Dirofilaria immitis*, in the pulmonary artery, the vessel that showed the most marked healed lesions. This made the interpretation of the healed lesions difficult. In dog no. 40-68 no "heart worms" were present yet the lesions were similar in all respects except those related to differences in survival time.

Discussion

The chief significance of these experiments is that they eliminate from an essential rôle one of the most puzzling features connected with these studies on "experimental necrotizing arteritis in dogs," namely, "plasma alteration." The original conjecture about the pathogenesis of these arterial lesions, "that they may be due to a hypersensitivity to some constituent of the blood of one or more of the donor dogs," has to be modified. The problem apparently becomes one of disturbed metabolism and resolves itself into two factors: (1) a dietary factor and (2) a factor associated with renal insufficiency.

This concept of the problem brings these studies into closer correlation with those of Winternitz and his co-workers,[5, 6] and with those

of Goldblatt [8] and others. The factor common to all of this experimental work is renal insufficiency, a factor long suspected of playing a part in arterial disease in humans. Brief evidence for a possible etiological relationship between renal injury and periarteritis nodosa has been given in the preceding paper.

At the present time it seems to make little difference as to how the renal insufficiency is produced; *i.e.*, by heavy metals, by constricting the renal arteries, by subtotal ureteral obstruction, or by extirpating the kidneys. With constriction of the renal artery, hypertension ensues, and this may be responsible for a necrotizing process affecting the arterioles rather than the larger arteries. Winternitz and Katzenstein [6] failed to observe any correlation between blood pressure and the anatomical lesions in their dogs, which involved arteries as well as arterioles. The few scattered determinations of blood pressure which I have made have not yielded any definite results.

Neither Goldblatt [8] nor Winternitz and his co-workers [5, 6] have found it necessary to control the diet of their animals. In my experiments to date, diet seems to play a definite rôle. Evidence for a dietary factor is admittedly incomplete but is accumulating and will be presented in the near future.

Summary

1. In all of my previous publications on "experimental necrotizing arteritis in dogs," [1-3] three factors have been constant:

a. Maintenance on a standard low protein diet during the course of the experiment (3 to 6 weeks).

b. Alteration of the blood plasma proteins—hyperproteinemia in 11 dogs by repeated injections of plasma obtained from healthy donor dogs; hypoproteinemia by plasmapheresis in 2 dogs.

c. Renal insufficiency—uranium nitrate in 8 dogs; [1] mercuric chloride in 2 dogs; [2] bilateral nephrectomy in 3 dogs.[3]

2. In the present report none of the dogs was subjected to any alteration of the blood plasma proteins but there was prolonged maintenance on the standard diet before renal injury was produced. Four of 5 dogs, maintained on the standard diet for 3 to 5 months and then subjected to renal injury (2 by uranium nitrate and 2 by bilateral nephrectomy), showed typical necrotizing arterial lesions when they died or were sacrificed 4 to 35 days later. The fifth dog, which survived bilateral nephrectomy only 3 days, showed suggestive early changes but no definite necrosis.

3. Prolonged maintenance on the standard diet is as effective as less prolonged maintenance on the diet *plus* "plasma alteration."

4. These experiments eliminate from an essential rôle one of the most puzzling features connected with these experimental arterial lesions, namely, "plasma alteration," but do not explain how "plasma alteration" augments the process.

5. The original conjecture "that the lesions may be due to a hypersensitivity to some constituent of the blood of one or more of the donor dogs" [1] is no longer tenable. The problem apparently becomes one of disturbed metabolism and resolves itself into two factors: (1) a dietary factor and (2) a factor associated with renal insufficiency.

REFERENCES

1. Holman, R. L. Acute necrotizing arteritis, aortitis, and auriculitis following uranium nitrate injury in dogs with altered plasma proteins. *Am. J. Path.*, 1941, **17**, 359–375.
2. Holman, R. L., and Hewitt, W. C. Experimental necrotizing arteritis. II. Mercuric chloride as effective as uranium nitrate in its production. *Proc. Soc. Exper. Biol. & Med.*, 1942, **49**, 58–62.
3. Holman, R. L. Experimental necrotizing arteritis in dogs. III. Bilateral nephrectomy as effective as heavy metal injury in its production. *Am. J. Path.*, 1943, **19**, 147–157.
4. Donnelly, G. L., and Holman, R. L. The stimulating influence of sodium citrate on cellular regeneration and repair in the kidney injured by uranium nitrate. *J. Pharmacol. & Exper. Therap.*, 1942, **75**, 11–17.
5. Winternitz, M. C.; Mylon, E.; Waters, L. L., and Katzenstein, R. Studies on the relation of the kidney to cardiovascular disease. I. *Yale J. Biol. & Med.*, 1940, **12**, 623–679.
6. Winternitz, M. C., and Katzenstein, R. Studies on the relation of the kidney to cardiovascular disease. II. *Yale J. Biol. & Med.*, 1940, **13**, 15–38.
7. Whipple, G. H. Personal communication.
8. Goldblatt, Harry. Experimental hypertension induced by renal ischemia. *Harvey Lectures*, 1937–38, **33**, 237–275.

DESCRIPTION OF PLATES

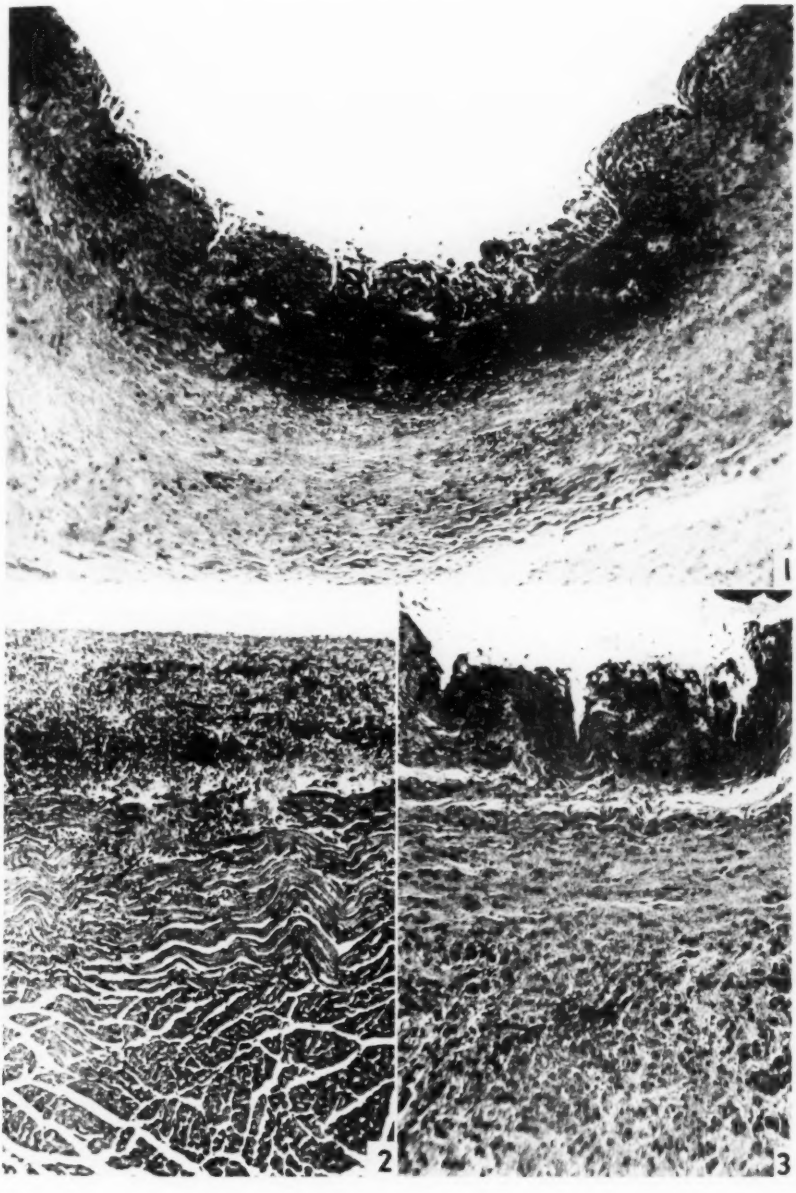
PLATE 21

Necrotizing arterial lesions in dogs. All sections were taken from dog no. 40-84 and were stained with hematoxylin and eosin.

FIG. 1. Acute necrotizing arteritis at point of origin of innominate artery from aorta. × 100.

FIG. 2. Acute necrotizing left auriculitis. × 100.

FIG. 3. Acute necrotizing arteritis of pulmonary artery. × 100.



Holman Experimental Necrotizing Arteritis. IV.

PLATE 22

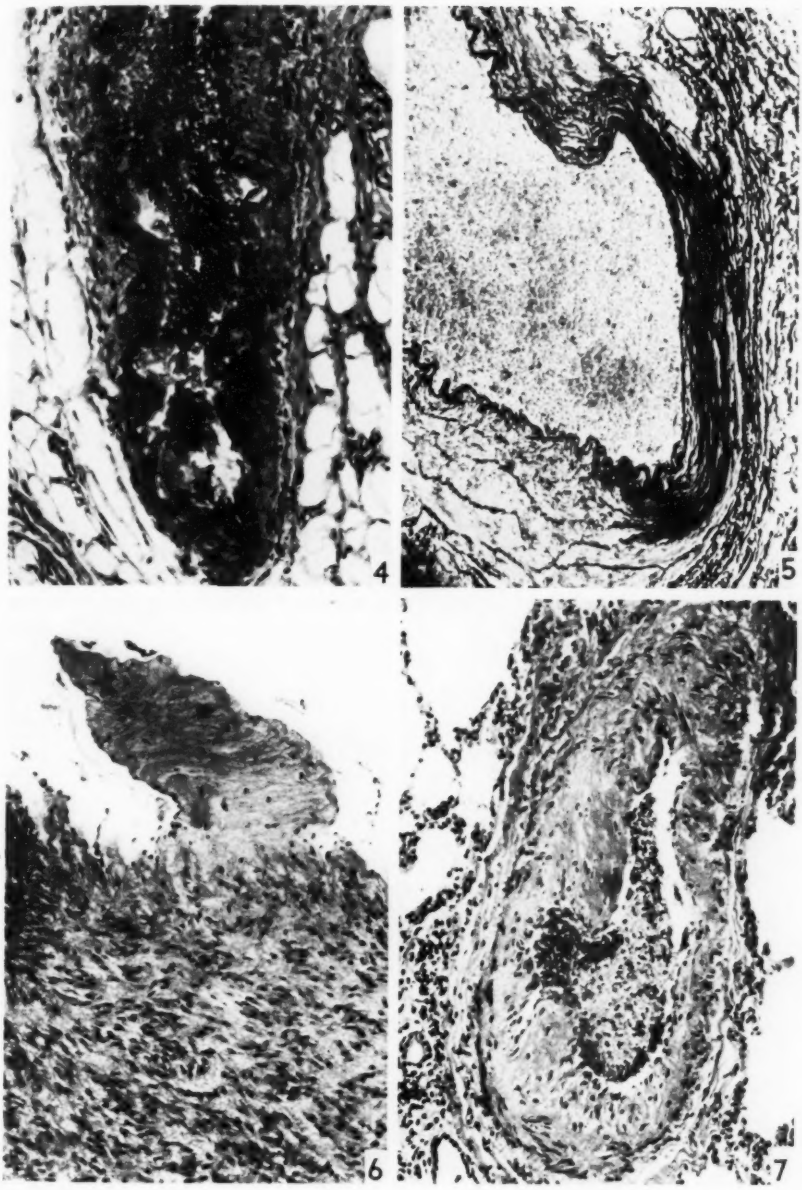
Necrotizing arterial lesions in dogs. All sections, except that used for Figure 5, were stained with hematoxylin and eosin.

FIG 4. Dog no. 40-79. Acute necrotizing arteritis of common carotid artery. × 128.

FIG. 5. Dog no. 40-79. Another section of common carotid artery, stained with Verhoeff's elastic tissue stain. The "normal" undulating pattern of the internal elastic membrane and medial elastic tissue is replaced with "straight lines." × 128.

FIG. 6. Dog no. 40-68. Healed lesion in intima of pulmonary artery. × 128.

FIG. 7. Dog no. 40-68. Healed lesion in artery in lung. × 150.



Holman Experimental Necrotizing Arteritis. IV.

STUDIES ON EXPERIMENTAL RICKETS IN RATS

IV. The Relation of Rickets to Growth, With Special Reference to the Bones *

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In former papers (1934, 1938 and 1939) we have described the changes in the bones of rachitic rats, with special reference to the epiphyseal cartilage, both in the active phase of rickets and during healing of the disease. Inasmuch as the osseous changes in these rats have been studied and described in considerable detail, it seems desirable to present our observations upon the growth pattern of these same rats, as it is modified during the development and healing of rickets, with special emphasis upon the growth of the bones. It is well known that experimental rachitogenic diets inhibit growth to varying extents. The observations recorded here give further information concerning growth as affected by the Steenbock-Black diet 2965, so widely used in experimental work. A significant feature of the present studies lies in the fact that we have traced the progress of rickets and the growth of the bones in individual rats by the use of frequent roentgenograms of the living rats.

Material and Methods

Rickets was produced by the Steenbock-Black rachitogenic diet 2965. Healing was produced in most of the rats by irradiated ergosterol (Viosterol), though in a smaller number cod-liver oil was used. The rachitogenic diet was begun at the age of 4 weeks; the curative treatment at 8 or 9 weeks of age, after rickets had become well established.

Growth records were made of 135 rats sacrificed for microscopic study at ages of 4 to 13 weeks, including 22 rats on a balanced ration and 113 on the rachitogenic diet. The records included 678 roentgenograms of the 135 rats. Of 58 rats roentgenograms were taken weekly or more often; from the remaining 77, at less frequent intervals or only at the time of sacrifice. The microscopic studies covered the bones from 112 of these rats. Line test studies were made of 37.

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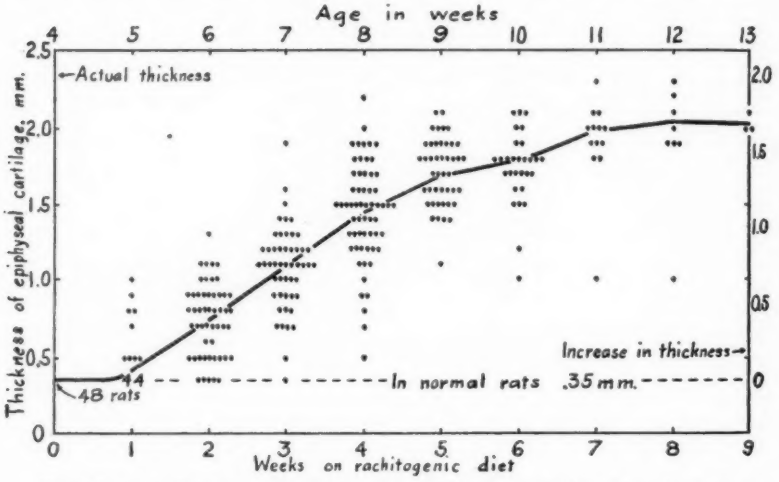
The roentgenograms were taken of entire rats as they lay, ventral side down, relaxed under light ether anesthesia. Measurements made on x-ray films under magnifications of a few diameters were found to be fairly accurate and served to show general trends of growth very well, though there were some minor irregularities due, presumably, to inaccuracies in measurement, or to varying attitudes of the bones. Certain corrections were made to compensate for the angles between the several bones and the photographic film. The length of the ver-


Age in weeks
4 5 6 7 8 9 10 11 12 13
Thickness of epiphyseal cartilage, mm.
2.5 2.0 1.5 1.0 0.5 0
←Actual thickness
Increase in thickness→
In normal rats .35 mm.
44
48 rats
0 1 2 3 4 5 6 7 8 9
Weeks on rachitogenic diet

Text-Figure 1. Scattergram showing rate of development of rickets in rats as measured by the increase in thickness of the epiphyseal cartilage in the head of the tibia, based upon 90 rats on the Steenbock-Black diet 2965 (359 separate x-ray records). The individual points represent weekly records. The curve shows the average value for each week.

tebral column is the least reliable of the measurements, because the vertebral column is subject to varied degrees of arching for which there is no obvious correction.

We have recorded graphically the growth of each rat, using appropriate arbitrary scales which make the several items readily comparable and at the same time show the actual values as measured. Text-Figures 2–4 show 3 rats with different habits of growth.

The Rate of Development of Rickets

As an index of the severity of rickets we employed the pathologic thickening of the epiphyseal cartilage in the head of the tibia, a widely used and easily measured anatomic feature of the disease. The weekly values for the thickness of this cartilage were plotted for 90 rats, using 359 roentgenograms, 261 of which showed some thickening of the cartilage (Text-Fig. 1). This graph shows the average rapidity

of development of rickets and the extremes for each week. In only 9 of 53 rats was there any measurable thickening after 1 week on the rachitogenic diet, whereas all but 5 showed at least slight thickening after 2 weeks. The curve shows that the thickening progressed rather rapidly in the earlier weeks, but more slowly in the later weeks, until a maximum of about 2 mm. was attained. Records for each individual rat showed the same general trend, although there were differences in the exact form of the curves.

This graph (Text-Fig. 1) does not definitely answer the question whether the cartilage wholly ceased to thicken, or merely grew very slowly during the later weeks. The individual records, however, indicated that in some rats the cartilage definitely continued to increase in thickness for 8 or 9 weeks on the diet, while in others there was no measurable increase after about the fifth week. Nor has it been clearly demonstrated that mitosis of the cartilage cells wholly ceased in some rats with advanced rickets, although our microscopic studies showed that it at least became very slow. The uncalcified condition of the thickened cartilage clearly allows some compression (emphasized by Park, 1939), which would prevent actual thickening even though there might be a slow multiplication of cells.

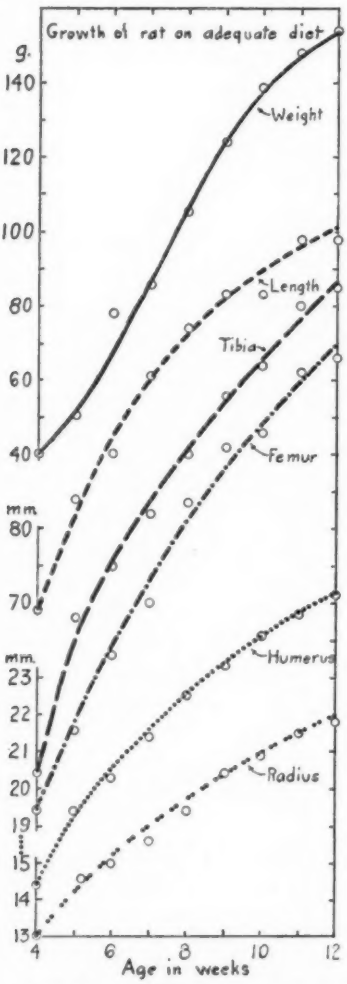
The few scattered records well below the main group in Text-Figure 1 represent a few rats with very mild rickets. Microscopic study showed that in these rats, calcification did not wholly cease, nor was cartilage removal entirely suspended. Cartilage removal did, however, lag considerably behind growth, so that the cartilage became somewhat, but not greatly, thickened. The thinner cartilage in such cases of mild rickets does not indicate less rapid cartilage growth than in severe rickets but, on the contrary, more rapid growth as shown by the observation that the elongation of such bones was definitely greater than in rats with severe rickets. Unfortunately, no weekly roentgenograms of these rats were taken, but only the terminal picture, as seen at the time of their sacrifice.

Rickets in Relation to Growth

The Weight of Rachitic Rats

The rats on the rachitogenic diet gained weight less rapidly than did the controls, an observation in accord with general experience with this and other commonly used rachitogenic diets (Text-Figs. 2, 3 and 5). In general it may be said that the more severe the rickets, the more the growth curve, after 5 or 6 weeks, fell below the normal. But the rats in which rickets developed most rapidly for the first 3 or 4 weeks were neither the heaviest nor yet the lightest, but rather the

intermediates. This relation is shown when the severity of rickets (thickening of the epiphyseal cartilage) is plotted against the gain in weight for rats of a given age (*e.g.*, at 7 weeks, as in Text-Fig. 6). This scattergram shows for the main group of rats an evident, positive correlation; that is, the rats which showed the greatest gains in weight since the beginning of the rachitogenic regimen had developed the most advanced rickets. But there was a minority, including rats much heavier than the main group, in which rickets was less advanced.

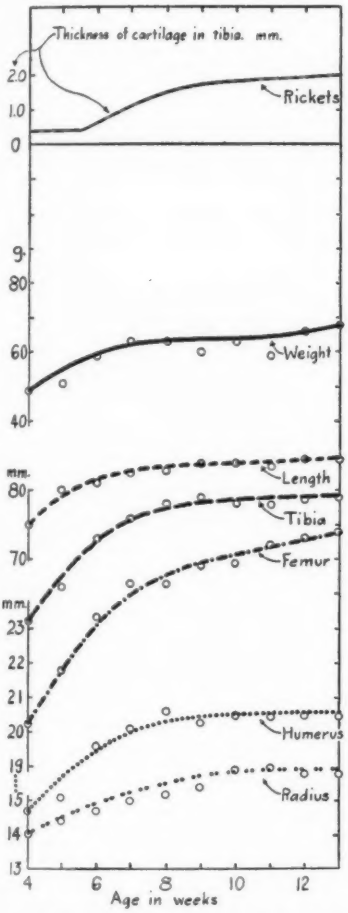


Text-Figures 2-4. Graphs of 3 rats showing typical modes of growth for weight, length of body and length of leg bones. Weekly records are shown by circles; growth trends by smooth curves. At the top of Text-Figures 3 and 4 are shown the development of rickets (as measured by the thickness of the epiphyseal cartilage) and the progress of healing (as measured by Bourdillon's 12-stage scale). All of these values are plotted to approximate arbitrary scales which make the separate items readily comparable.

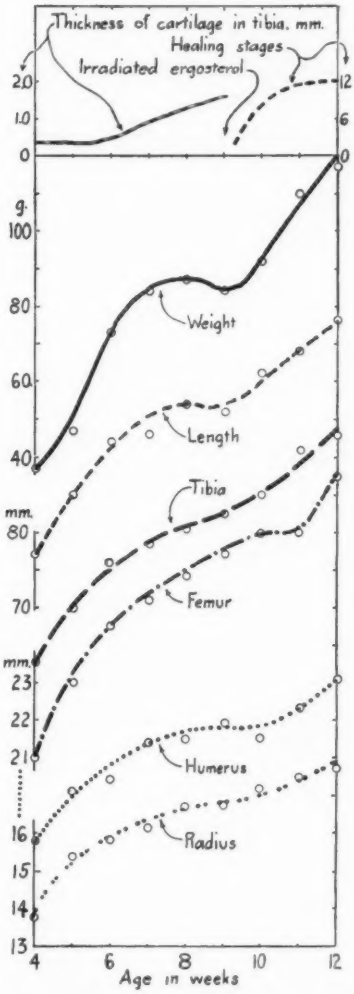
Text-Figure 2. Rat no. 130, male, maintained on a balanced diet. The growth is well within the limits for normal, healthy rats.

This peculiar relation was shown equally well in these rats after 2, 3, or 4 weeks on the diet.

But when for these same rats the growth of the leg bones (*e.g.*, the



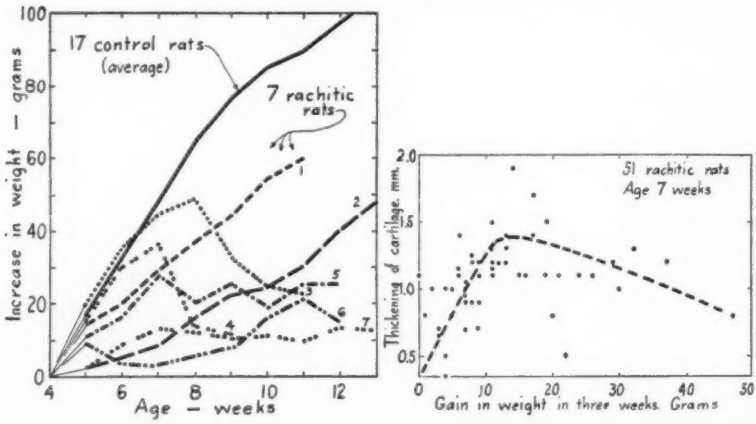
Text-Figure 3. Rat no. 155, male, maintained on diet 2965. Persistent, severe rickets. Growth was somewhat subnormal even during the first week on the experimental diet, and almost ceased after 5 weeks.



Text-Figure 4. Rat no. 134, female, maintained on diet 2965 throughout, but given irradiated ergosterol after 5 weeks. After about 5 weeks on the rachitogenic diet the growth rate became much reduced, but it was accelerated when healing was produced by the administration of vitamin D.

tibia) was plotted against the rate of development of rickets, all the records fell in a compact group showing a direct correlation. The heavy rats shown in Text-Figure 6 were evidently those which were able, to a high degree, to resist the development of rickets and to escape the degree of emaciation which accompanied rickets in the others.

The increase in weight of rachitic rats did not follow any definite pattern, but assumed a wide diversity of forms (Text-Fig. 5). The growth patterns were not distributed at random, but all rachitic mem-


17 control rats (average)
7 rachitic rats
Increase in weight grams
Age — weeks
51 rachitic rats Age 7 weeks
Thickening of cartilage. mm.
Gain in weight in three weeks. Grams

Text-Figure 5. Graphs showing average increase in weight of 17 normal rats and of 7 individual rachitic rats (no curative agent fed and no spontaneous healing). These rats illustrate the variety of growth patterns assumed by rachitic rats. Each one is typical of a considerable number of rats of both sexes.

Text-Figure 6. Scattergram of 51 rats (age 7 weeks) showing relation between gain in weight and severity of rickets after 3 weeks on diet 2965. About 40 of the rats fall in a group showing positive correlation between gain in weight and severity of rickets (thickness of epiphyseal cartilage). The remaining heavy rats suggest a reverse correlation.

bers of a litter usually grew similarly, as indeed did those in all litters at about the same time. The growth pattern changed from time to time, but there was not observed any pattern definitely characteristic of any season of the year. In general, in accord with common experience, rickets was more severe and the growth less rapid in winter than in summer, but this was by no means a clear-cut rule. Zucker, Hall and Young (1941), in a recent paper, emphasized these "seasonal or recurrent, aperiodic variations" which must be considered in making any kind of comparative growth study upon rachitic rats. Such variations have made it necessary to use great caution in drawing conclusions from our averages.

The reason for such variations in growth response to the rachitogenic diet is not evident. Temperature may be a minor factor, but rats kept at abnormally high temperature did not yield any definite information concerning the effect of temperature. In respect to the severity of rickets in relation to temperature, Tourtellotte and Bacon (1935) are not in agreement with the conclusions of Guerrant, Dutcher and Crowthers (1937), which would suggest that there cannot be any considerable influence of temperature. Nor were the differences in growth patterns distributed according to sex; the females grew less rapidly than the males, but the various patterns were distributed between the two sexes. Nor did the growth pattern bear any detectable relation to the weight of the young rats at the beginning of the experimental period, nor to the previous diet of the mothers. Presumably some sort of undetected differences in the rachitogenic diet must be responsible.

The Growth of the Leg Bones

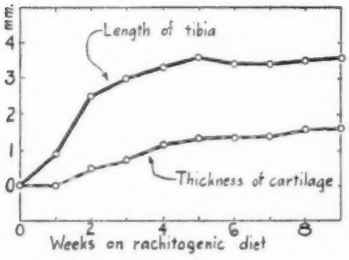
The growth of the leg bones was retarded greatly in rachitic rats. In a given rat all the leg bones followed the same general pattern and all were retarded to about the same extent (Text-Fig. 3). The bones of different rats did not show the same diversity of growth patterns as did the weights of the same rats, possibly largely because the length of bones cannot be subject to the same fluctuations as the weight of the animal. The only conspicuously different growth patterns of bones were those characteristic of severe rickets, mild rickets and healing rickets (Text-Figs. 8, 9 and 10).

The weekly measurements showed that the bones of rachitic rats suffered progressive retardation until, after about 4 weeks on the diet, the growth became very slow, and in some rats ceased entirely. It is clear, however, that there was no definite and universal growth limit at the age of 7 weeks (3 weeks on the diet) as there seemed to be from our earlier estimations which were not checked by weekly roentgenograms (1934), nor at about 11 days as recorded by Park (1939).

The Tibia and Its Epiphyseal Cartilage

The growth of the tibia and its epiphyseal cartilage gives a clear illustration of the way in which rickets influences the elongation of bones through its effect on the epiphyseal cartilage. In rats over 4 weeks of age only the cartilage in the proximal end of the bone need be considered, because by that time the cartilage in the distal end has ceased to be a factor in the growth of the bone.

When the elongation of an average rachitic tibia and the thickening of its epiphyseal cartilage were plotted to the same scale and from a common origin, the two curves diverged for a time, but soon became nearly parallel, and throughout the greater part of their courses were separated by about 2 mm. (Text-Fig. 7). During the first week the tibia showed little effect of rickets and continued to grow in length



Text-Figure 7. Rat no. 155, male, with persistent severe rickets. The curves for the growth of the tibia and for the increasing thickness of the epiphyseal cartilage are plotted from a common origin. The two curves are divergent until cartilage removal ceases (for about 2 weeks), after which they become nearly parallel, being separated by about 2 mm. The parallel relation persists as long as severe rickets continues.

about as in normal bones. During these days the cartilage became no thicker, because its growth was normally utilized in the production of bone in the end of the growing shaft. But when the advance of calcification into the cartilage ceased and cartilage removal was suspended, the calcified shaft of the bone also ceased to grow longer. The uncalcified cartilage, however, continued to grow and, because none of it was being removed, increased in thickness. Thus it comes about that the elongation of severely rachitic bones is expressed wholly in the increased thickness of the cartilage rather than in the normal elongation of the osseous shaft. The parallel course of the two curves in Text-Figure 7 shows this relation.

The growth relations shown in Text-Figure 7 represent about the average condition in severe rickets. The departures from this average condition involve a time factor (varying time elapsing before calcification and cartilage removal begin to lag behind growth, and before these two processes wholly cease); and a growth factor (varying rates of growth). The time factor determines how soon the curves become parallel; the growth factor, the distance between their parallel portions.

On the average, the tibia had grown in length 1.5 mm. (extremes of 0.5 mm. and 2.7 mm.) before the cartilage began to thicken; and had grown 2.0 mm. (extremes of 1.5 mm. and 3.0 mm.) before calcification and cartilage removal wholly ceased. The continued thickening of the cartilage after the cessation of its removal added an average of about 1.7 mm. to the length of the bone, a value from which the variations in either direction were not great. Thus, in rats with severe and persistent rickets, the total average growth of the

tibia during the rachitogenic regimen was 3.7 mm. (2.0 mm. plus 1.7 mm.), with extremes of 3.2 mm. and 4.7 mm. This is about one-third of the normal growth, inasmuch as during the same weeks (up to the age of 12 weeks) the average growth of the tibia in the control rats was about 10 mm. (Text-Fig. 10). The behavior of the tibia as described above may be considered as characteristic of the long bones in general.

The Growth of the Vertebral Column

The growth of the body in length (the growth of the vertebral column as measured from the base of the skull to the caudal end of the innominate bone) showed deficiency, as did the leg bones (Text-Figs. 2, 3 and 9), though the varied amount of arching, especially pronounced in rats with very severe rickets, made roentgenograms unreliable in the detection of small increments of growth. In the control rats the vertebral column continued to grow steadily, until at the age of 12 weeks it had made an average growth of 47 mm. over its length at 4 weeks. In the rachitic rats the growth of the vertebral column showed progressive retardation, as did the leg bones. After about the ninth week its growth was very slight, and by the end of the twelfth week it showed an average increase of only 12 mm. since the fourth week (about one-fourth the normal growth).

During the experimental period (4 to 12 or 13 weeks of age), the leg bones of the control rats grew relatively less rapidly than did the vertebral column, so that the legs became proportionally shorter. In our rachitic rats the relative retardation of the vertebral column was greater than that of the leg bones, to the extent that in them the legs and the vertebral column accomplished about the same relative growth.

It is not surprising that rickets should affect the growth of the legs and the vertebral column to different extents, because the growth of each leg is the result of rapid growth of very few cartilages (two, exclusive of those in the foot), whereas the growth of the vertebral column is the summation of the growth of sixty cartilages, each of which grows very slowly. It is conceivable that rickets might affect the two types of cartilage to different extents, but it is not evident why the retardation should be greater in the cartilages which normally grow slowly than it is in those which grow rapidly. In this connection it is of interest to note that in two series of rats on two different non-rachitogenic diets (Outhouse and Mendel, 1933) the leg bones in the more slowly growing series were longer with respect to the body than in those which grew more rapidly, a result similar to that observed in our rachitic rats.

The Healing of Rickets in Relation to Growth

Measuring the Healing Process

The degree of healing of rickets, as commonly measured by the line test, is an indication of the extent of resumed calcification. This calcification is intimately associated with the extensive tissue reorganization of the healing process (Dodds and Cameron, 1938). Roentgenograms, although not as extensively used as the line test, afford another very useful means of observing the extent of calcification, and have the advantage of applicability to the living rat. Some writers have advocated their wider use (Poulsson and Lövenskiold, 1928; Bourdillon, Bruce, Fischmann and Webster, 1931), and have described useful methods. Our estimation of healing is based upon the 12-stage scale of Bourdillon and co-workers (Dodds and Cameron, 1938).

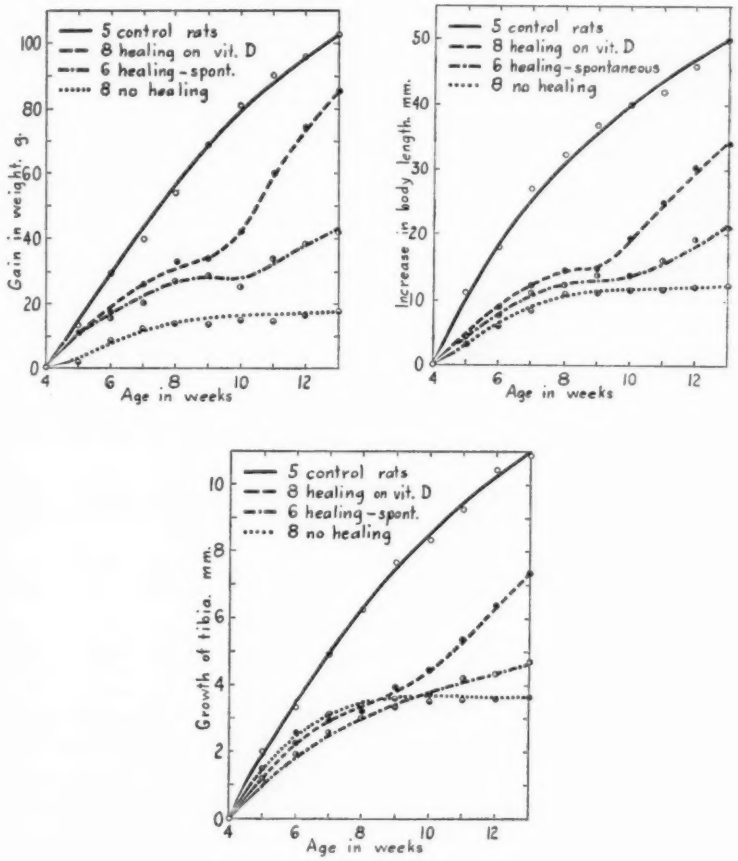
Graphs from roentgenograms of the head of the tibia showed that healing began promptly after the administration of vitamin D, and that it was often practically complete in 2 weeks, although a longer time frequently was needed. Text-Figure 4 includes graphs showing the progress of healing in a typical rat under the influence of vitamin D. The roentgenograms also revealed cases of spontaneous healing which began without the use of vitamin D. No such case of spontaneous healing came to completion in any observed rat, but after a week or two gave place to renewed incidence of rickets. We interpret these cases of spontaneous healing as representative of an intermediate condition between severe rickets and the mild form in which cartilage removal does not wholly cease.

Healing and Accelerated Growth

When healing took place under the influence of vitamin D there was a marked improvement in growth, both in the weight of the rats and in the length of the bones. Comparison of Text-Figures 3 and 4 shows resumed growth in a typical rat. A comparable acceleration was seen in every rat which was allowed to live long enough to bring healing to practical completion.

We have recognized and plotted four general types of growth, the types corresponding to the severity of rickets (Text-Figs. 8–10). These types are distinguishable with equal clearness with respect to increase in weight, growth of the leg bones and growth of the vertebral column. These types are as follows: (1) Normal growth seen in rats on a balanced ration. (2) Serious and persistent growth retardation seen in those rats on the rachitogenic diet which developed severe and persistent rickets. (3) Marked retardation followed by acceleration

in the later experimental weeks, seen in rats which were undergoing healing under the influence of vitamin D. Not all rats undergoing healing on vitamin D showed as great growth acceleration as the eight included in the average shown in these figures, but it is true that every such rat showed more rapid growth than those in the same litters which did not receive the vitamin D. (4) An intermediate group vacillated between severe rickets and spontaneous partial heal-



Text-Figures 8, 9 and 10. Average growth curves for normal rats and for rats showing three different degrees of rachitic severity (compare Text-Figs. 2-4). Normal rats grew steadily and rapidly. Rats with persistent rickets grew with increasing slowness until growth almost ceased. Rats with spontaneous healing grew slowly but showed a tendency to acceleration in the latter weeks. Rats under the influence of vitamin D showed marked acceleration after healing took place. Compare Text-Figure 8, increase in weight; Text-Figure 9, growth of vertebral column; and Text-Figure 10, growth of tibia.

ing, with recurrent rickets. The growth trend was also intermediate between those of the two preceding groups of rachitic rats.

To these four might be added a fifth group, including a few rats with very mild rickets (shown in Text-Fig. 1), in which calcification was less seriously deficient and removal of the epiphyseal cartilage was never wholly interrupted. The growth of these rats was only moderately retarded (Text-Fig. 5, curve 1). There were no weekly roentgenograms of these rats, but the final picture shows that the bones accomplished distinctly greater growth than in the other rachitic groups.

Discussion

Inasmuch as growth is much retarded when experimental rickets develops in rats and is resumed when healing takes place, the conclusion might be drawn that reduced growth is part of the rachitic condition. On the other hand, when vitamin D is added to the rachitogenic diet 2965 throughout the experimental weeks, though no anatomic evidences of rickets develop, yet the growth is no better than in rats receiving diet 2965 alone. This result has been observed in our rat colony and has been pointed out also by Kunde and Williams (1927) and by Zucker, Hall and Young (1941) and others. Such results might suggest that the growth deficiency is due directly to diet deficiency rather than to rickets. But the answer is not as simple as that.

It has been observed by various workers (among them Osborne and Mendel, 1918) that inadequacy of phosphorus is a common cause of reduced growth; and more recently it has been pointed out that phosphorus deficiency is the chief growth-limiting factor of the rachitogenic diet 2965 and other high-calcium, low-phosphorus diets (Shohl and his co-workers, 1928, 1933 and 1939, and other workers). When renewed calcification takes place in the healing of rickets under the influence of vitamin D, it is presumably the better utilization of the scant phosphorus in the ration that makes possible the more rapid growth of all bodily tissues. But it is not evident why the rachitogenic diet with vitamin D added, though preventing rickets, does not produce better growth than the rachitogenic diet alone. This is especially puzzling in consideration of the marked growth acceleration which accompanies healing under the influence of vitamin D.

Day and McCollum (1939) and Follis, Day and McCollum (1940) found that with a purified rachitogenic diet, very low in phosphorus, the very grave growth deficiency was due, in part at least, to inanition, because the rats did not eat enough of the ration. We found that in

our colony the rats on the rachitogenic diet did consume a little less food than those on the stock diet, but we observed no evidences of inanition. Moreover, the rachitic rats actually consumed more food per unit of body weight than did the controls. The quality of the food rather than the quantity was responsible for the deficient growth.

Inasmuch as phosphorus deficiency is known to produce both rickets and subnormal growth, it would seem that the growth deficiency should be considered a part of low-phosphorus rickets. It is generally agreed that vitamin D produces an increased net absorption of phosphorus from the alimentary tract of rachitic rats which is sufficient to relieve the rachitic condition and to bring about somewhat better growth. But as long as the phosphorus in the ration is markedly low, the improvement in rachitic rats does not result in fully normal growth nor, according to Zucker, Hall and Young (1941), in a completely normal mineral content of the bones.

Vitamin D is not of itself a growth-promoting agent; it merely acts to increase the available phosphorus. Of interest in this connection are the results of Stearns, Jeans and Vandecar (1936) and Slyker, Hamil, Poole, Cooley and Macy (1937), who observed in over 500 infants an increase in growth rate upon the addition of vitamin D to a ration already adequate enough to prevent rickets. Presumably the vitamin D was effective in increasing growth through its power to bring about greater economy of the phosphorus in the ration.

The growth modifications observed microscopically in the rachitic epiphyseal cartilage (decreased cell multiplication and reduced growth of the cells in the columns) would indicate that in rickets there must be a slowing of growth of the bones, quite apart from actual gross measurements of this deficiency (Dodds and Cameron, 1934). Since the observed deficiency in growth takes place in the zones of the cartilage which even in normal bones are not calcified, one is led to wonder if the subnormal weight of the animals may not be due to a similar effect upon other noncalcified tissues. This growth-limiting effect upon the epiphyseal cartilages of bones is quite distinct from the commonly recognized phase of rickets (inadequate calcification of bone and cartilage) and constitutes a separate phase of the disease, presumably also brought about directly by the phosphorus scarcity. This limitation of cartilage growth has not been recognized by most students of rickets, and the excessive accumulation of uncalcified cartilage has led some to the erroneous conclusion that there is an actual acceleration in cartilage growth.

Investigators in the field of experimental rickets have generally recognized that in rachitogenic diets the scarcity of phosphorus is

not the only growth-limiting deficiency, though the others have not been so definitely defined. For this reason the growth deficiency of rats receiving the diet 2965 probably is greater than might be expected in uncomplicated low-phosphorus rickets. The existence of these other deficiencies in this diet has recently been clearly demonstrated by Zucker, Hall and Young (1941), who found that the addition of adequate amounts of phosphate to this ration still left it quite inadequate to produce normal growth. In an attempt to find a better rachitogenic diet, these workers have devised a high-calcium, low-phosphorus diet, no. 803, which produces as severe rickets as diet 2965 with less retardation of growth. Moreover, when phosphate in proper amount is added to diet 803, it becomes practically a complete diet and produces very nearly normal growth in rats. But like diet 2965, this diet does not show improved growth production upon the addition of vitamin D alone.

Though it is generally recognized that growth deficiency accompanies experimental rickets in rats, there has been no general agreement as to the impairment of growth in rachitic human infants. Hess (1929) stated that in the common moderate case of human rickets there is usually no decrease in the growth rate, though in the extreme form of rickets impairment of growth may be encountered. He quoted Wimberger (1923), who studied the growth of the tibia and other long bones by roentgenograms, and came to the conclusion that growth does not suffer any impairment through rickets.

On the other hand, we find statements like that by Mueller (1924), who said it is well known that in high-grade rickets, growth in length suffers to a conspicuous degree. Feer (1922) stated that rachitic children are always shorter than others, even if there is no evident distortion of the bones. Holt and Howland (1929) said: "A change which has not been sufficiently emphasized is arrested growth of the long bones; this is the most characteristic feature of rickets." Reed, Struck and Steck (1939) emphasized the importance of even very mild rickets because of the depressing effect upon growth. Indeed, the studies of Slyker and co-workers and of Stearns, Jean and Vandecar seem to indicate that infants suffer growth deficiency when there is an inadequacy of phosphorus which is not quite severe enough to produce rickets.

On the whole, it would seem that we must accept the view that rachitic infants commonly suffer growth deficiency. It is probable, however, that the deficiency is by no means as great as that observed in rachitic rats, otherwise there would scarcely be any question about its occurrence. No doubt the division of opinion may also be due in

part to the impossibility of carrying out extensive experimental studies under rigidly controlled conditions.

The seemingly different magnitude of growth response between rachitic infants and rats may possibly be explained in part by the different dietary requirements necessary for the production of rickets in the two species. Rats do not develop rickets unless, in addition to deficiency in vitamin D, there is also a distinctly unfavorable ratio of calcium and phosphorus in the diet, usually high-calcium and low-phosphorus (Hess, 1929; Shohl, Bennett and Weed, 1928; and others). Infants, on the other hand, commonly develop rickets on diets deficient only in vitamin D, or when deprived of solar radiations, even though calcium and phosphorus in the diet may be adequate. Zucker, Hall and Young (1941) expressed the situation by saying that in rats rickets is primarily a phosphorus deficiency (though it can be largely corrected by vitamin D), while in infants it is purely an inadequacy of vitamin D, usually in the presence of adequate calcium and phosphorus in the diet. Thus, infants often have rickets on a diet of cow's milk, whereas rats will not become rachitic when there is cow's milk in their diet. On such a diet infants may suffer physiologic scarcity of phosphorus, even though satisfactory amounts of phosphorus are actually presented in the food. The phosphorus scarcity so produced is seemingly usually not severe enough to retard growth as seriously as when rickets is produced experimentally in rats.

Summary

These studies are based upon 135 albino rats, in most of which rickets was produced by the Steenbock-Black diet 2965. The special feature of the study is the tracing of the development and healing of rickets and the growth of the bones by weekly roentgenograms. Graphic methods are used to show rickets, healing and growth.

The rachitic rats were very subnormal in weight, but their growth did not follow any single pattern.

The growth of the leg bones and of the vertebral columns of the rachitic rats was greatly retarded. The retardation of the vertebral columns was relatively greater than of the leg bones.

The epiphyseal cartilage of the tibia (typical for all long bones), during the first week or two on the rachitogenic diet continued to make its contribution to the length of the shaft of the bone, but in decreasing amount. After about the third week the shaft ceased to elongate, and the pathologic thickening of the epiphyseal cartilage and the elongation of the bone became equal and identical.

When rickets healed under the influence of vitamin D, both increase in weight and growth of bones were accelerated to a marked degree. When spontaneous healing took place there was a less marked acceleration.

We believe that the reduction in growth rate is a part of low-phosphorus rickets, and, like other phases of rickets, is produced by phosphorus deficiency. Other growth-limiting factors in most rachitogenic diets are also recognized, but these are not concerned in the production of rickets.

BIBLIOGRAPHY

Bourdillon, R. B.; Bruce, H. M.; Fischmann, C., and Webster, T. A. The quantitative estimation of vitamin D by radiography. *Medical Research Council, Special Report Series, No. 158,* His Majesty's Stationery Office, London, 1931.

Day, H. G., and McCollum, E. V. Mineral metabolism, growth, and symptomatology of rats on a diet extremely deficient in phosphorus. *J. Biol. Chem.,* 1939, **130,** 269–283.

Dodds, G. S., and Cameron, H. C. Observations on the growth rate of rachitic rats. (Abstract.) *Anat. Rec.,* 1934, suppl. **58,** 11.

Dodds, G. S., and Cameron, H. C. Studies on experimental rickets in rats. I. Structural modifications of the epiphyseal cartilages in the tibia and other bones. *Am. J. Anat.,* 1934, **55,** 135–165.

Dodds, G. S., and Cameron, H. C. Studies on experimental rickets in rats. II. The healing process in the head of the tibia and other bones. *Am. J. Path.,* 1938, **14,** 273–296.

Dodds, G. S., and Cameron, H. C. Studies on experimental rickets in rats. III. The behavior and fate of the cartilage remnants in the rachitic metaphysis. *Am. J. Path.,* 1939, **15,** 723–740.

Feer, Emil. Textbook of Pediatrics. (Tr. and ed. by J. P. Sedgwick and C. A. Scherer.) J. B. Lippincott Co., Philadelphia, 1922.

Follis, R. H., Jr.; Day, H. G., and McCollum, E. V. Histological studies of the tissues of rats fed a diet extremely low in phosphorus. *J. Nutrition,* 1940, **20,** 181–195.

Guerrant, N. B.; Dutcher, R. A., and Crowthers, Ruth. Environmental temperature as a factor in the production and in the cure of rickets in the rat. *J. Nutrition,* 1937, **14,** 471–480.

Hess, A. F. Rickets, Including Osteomalacia and Tetany. Lea & Febiger, Philadelphia, 1929.

Holt, L. E., and Howland, John. The Diseases of Infancy and Childhood. D. Appleton-Century Co., New York, 1929, ed. 9.

Kunde, M. M., and Williams, L. A. Experimental cretinism. II. The influence of the thyroid gland on the production and control of experimental rickets. *Am. J. Physiol.,* 1927, **83,** 245–249.

Lilly, C. A.; Peirce, C. B., and Grant, R. L. The effect of phosphates on the bones of rachitic rats. *J. Nutrition,* 1935, **9,** 25–35.

Mueller, Walther. Die normale und pathologische Physiologie des Knochens. J. A. Barth, Leipzig, 1924.

Osborne, T. B., and Mendel, L. B. The inorganic elements in nutrition. *J. Biol. Chem.,* 1918, **34,** 131–139.

Outhouse, Julia, and Mendel, L. B. The rate of growth. I. Its influence on the skeletal development of the albino rat. *J. Exper. Zoöl.,* 1933, **64,** 257–285.

Park, E. A. Observations on the pathology of rickets with particular reference to the changes at the cartilage-shaft junctions of the growing bones. *Harvey Lectures,* 1938–39, **34,** 157–213.

Poulsson, Edvard, and Lövenskiold, Herman. The quantitative determination of vitamin D. *Biochem. J.,* 1928, **22,** 135–141.

Reed, C. I.; Struck, H. C., and Steck, I. E. Vitamin D: Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations. University of Chicago Press, 1939.

Shohl, A. T. Physiology and Pathology of Vitamin D. In: The Vitamins. A Symposium. American Medical Association, Chicago, 1939, pp. 459–474.

Shohl, A. T.; Bennett, H. B., and Weed, K. L. Rickets in rats. VII. Metabolism of calcium and phosphorus of rats fed upon non-ricketogenic diets. *J. Biol. Chem.,* 1928, **79,** 257–267.

Shohl, A. T.; Brown, H. B.; Chapman, E. E.; Rose, C. S., and Saurwein, E. M. The evaluation of the phosphorus deficiency of the rickets-producing diet. *J. Nutrition,* 1933, **6,** 271–284.

Slyker, Francis; Hamil, B. M.; Poole, M. W.; Cooley, T. B., and Macy, I. G. Relationship between vitamin D intake and linear growth in infants. *Proc. Soc. Exper. Biol. & Med.,* 1937–38, **37,** 499–502.

Stearns, Genevieve; Jeans, P. C., and Vandecar, Verva. The effect of vitamin D on linear growth in infancy. *J. Pediat.,* 1936, **9,** 1–10.

Tourtellotte, D., and Bacon, W. E. Variability of vitamin D response with temperature of environment. *J. Nutrition,* 1935, **10,** 683–688.

Wimberger, Hans. Röntgenometrische Wachstumsstudien am gesunden und rachitischen Säugling. *Ztschr. f. Kinderh.,* 1923, **35,** 182–194.

Zucker, T. F.; Hall, Lilian, and Young, Margaret. Growth and calcification on a diet deficient in phosphate but otherwise adequate. *J. Nutrition,* 1941, **22,** 139–151.

A FATAL DISEASE OF MIDDLE-AGED MICE CHARACTERIZED BY MYOCARDITIS ASSOCIATED WITH HEMORRHAGE IN THE PLEURAL CAVITY *

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In routine autopsies of a large colony of mice maintained in this department for the study of leukemia, 107 mice were found to have died during a period of approximately 3 years with a well characterized disease complex still present in our colony which, to our knowledge, has not previously been described. Although we have been unable to determine the etiology of this disease, it seems desirable to describe it because it apparently constitutes a well defined entity that is of sufficient interest to warrant further investigation.

Incidence; Age and Sex Relationship

The colony is comprised of from 5000 to 7000 mice per annum, with a mortality of about 3000 per annum. The disease occurred throughout the year, affecting adult male mice. At the early phase of this study 5 female mice were recorded as having died of this disease, but the hearts of these animals were not sectioned, and since this disease has been studied carefully it has not been found among female mice. The age distribution was as follows:

Age in months	Less than 5	6–7	8–9	10–11	12–13	14–15	16–17	18–19	20–21	22–23	Over 23
Number of mice	4	4	8	16	16	19	12	13	10	2	1

This tabulation shows that the greatest mortality occurred between the 10th and 15th month, with a maximum at 14 months. The greater part of our colony is under 12 months of age. All of the last 14 cases observed occurred among males, and 13 were of the Rf stock. Their age varied from 8 to 19 months, with an average of 13 months.

Anatomic Changes

Gross examination indicated that exsanguinating hemorrhage into the pleural cavity was the immediate cause of death in most instances. Coexisting myocardial and epicardial hemorrhage was common, while the lungs were, as a rule, free from hemorrhage; the vascular rupture

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usually occurred near the origin of the great vessels. Peritoneal hemorrhage was noted in only 1 animal. Testicular hemorrhage, however, occurred frequently and was recorded in 27 mice. This figure, however, is inaccurate. More recently all testes have been sectioned and hemorrhage, although minute, is found in almost every case. Testicular hemorrhage unaccompanied by pleural hemorrhage was recorded at first in 11 cases, but in none of these was the heart examined microscopically; myocarditis was doubtless overlooked in several instances. More recently, the hearts have been examined microscopically in every case, and advanced myocarditis found, which may have accounted for the death of the animals. Thrombi distending the auricles of the unopened heart were noted in several cases.

The only other conspicuous change on gross examination was ischemia of the liver with yellowish discoloration, due to exsanguinating pleuropericardial hemorrhage, and moderate to advanced fatty degeneration. In many cases, the liver appeared smaller than normal. In general, the animals appeared to be in an excellent state of nutrition; many appeared obese and all were well developed.

Heart. Microscopic examination showed acute and subacute interstitial myocarditis in 23 of 25 mice that were examined. Since the bodies from the 2 mice without myocarditis have been discarded, it is impossible to state whether or not pleural hemorrhage in these cases was due to a different disease from that described here. The most frequent histologic picture was a diffuse infiltration by polymorphonuclear and mononuclear leukocytes of the muscle of auricles and ventricles (Figs. 1, 2 and 4), most extensive in the region of the auriculoventricular junction. The cardiac fibers were often widely separated by edema fluid. In areas where the inflammation was most intense, there was occasionally considerable necrosis of the heart muscle. In several animals, the process was extensive and a site of rupture was demonstrated in the auricular wall. The inflammatory process always involved the myocardium; the pericardum about the base of the heart was also frequently involved (Fig. 3), as was the endocardium, but to a lesser extent. The serosal and endocardial layers were elevated in several places by both inflammatory exudate and hemorrhage. Antemortem thrombi were occasionally attached to the auricular endocardium. The valve leaflets, as seen in sections occasionally, showed slight or moderate inflammation and hemorrhage. Frequently the myocardial changes were more chronic and characterized by considerable numbers of mononuclear leukocytes, lymphocytes and fibroblasts (Fig. 4). Occasionally, sheetlike masses of large mononuclear cells were seen, with "owl-eyed" nuclei, as in Aschoff

bodies. The cells were evidently Anitschkow "myocytes" [1] or "myocardial reticulocytes." [2] Characteristic Aschoff bodies were not seen. Fibrosis unassociated with inflammation was never observed.

Serial sections of the heart and lungs were made from 3 animals and the site of rupture was demonstrated in each. It was situated in either the auricle or in an adjacent orifice of a large vein. No parasite or micro-organism could be demonstrated with certainty.

Lungs. The most constant and conspicuous lesion was a hyaline degeneration of the smooth muscle in the walls of the medium-sized pulmonary arteries. It was observed in 11 of 27 mice. This alteration in the vessel was not associated with hemorrhage. A moderate degree of hyaline degeneration of arteries was observed in the lungs of only 3 of 26 control mice of the same age and sex. Chronic bronchitis and bronchiectasis were observed frequently, but the incidence was not greater than in the controls. Rarely, numerous erythrocytes were seen in the alveoli.

Testes. Extensive hemorrhage between the seminiferous tubules was a characteristic occurrence. Grossly, as a rule, only one testis seemed involved, but on microscopic examination the hemorrhage was usually bilateral (Fig. 5). There was frequently a slight degeneration of the tubules, but spermatogenesis was not prevented. Often small or moderate numbers of polymorphonuclear leukocytes were present; this was not a constant feature, and a few polymorphonuclears were also found in sections of testes with no hemorrhage. No rupture of any blood vessel was demonstrated with certainty although in 4 instances moderate degeneration was observed in the walls of the blood vessels. In several cases there was calcification of the media of large arteries in both capsule and parenchyma of the testes (Fig. 6). There seems to be no relation between this medial calcification and the acute fatal illness. While the cardiac lesions often indicated a subacute process, those of the testes were always acute.

Other Organs. The kidneys, spleen, adrenals, bone marrow, hypophysis and brain from a representative group of mice dead with this disease complex were examined. No constant pathologic change was noted in these organs.

Epidemiologic Observations

The mouse colony occupies four large rooms on two floors. Four different inbred stocks of mice and hybrids were studied. The disease occurred in all stocks and hybrid mice, and its incidence was roughly proportional to the number in each stock. Thirty mice with this disease were of the Rf stock and only 3 of stock Ak, but most mice of the

latter stock die within approximately 10 months, mainly of leukemia, whereas the peak of the mortality curve among Rf mice is at 15 months. Thus, many more mice of the Rf stock reached the period of susceptibility to the disease studied. Similarly, most hybrid mice are long-lived and most examples (63) of this disease were found among hybrids.

To obtain information on the possible spread of the disease in the animal colony, the cages in which mice had died with this disease were marked without changing their location and without sterilization. There was no evidence to indicate spread of the disease within the cage or from cage to cage, although several cases occurred in the same cage at widely varying intervals.

Bacteriologic Examination

The hearts from 8 cases of myocarditis were ground and cultured on the usual laboratory media. Inoculations were also made on 30 per cent horse serum agar for the detection of pleuropneumonia-like organisms; however, none were isolated. The organisms grown from a few cases were usually *Staphylococcus albus* and Gram-negative bacilli. These were regarded as postmortem invaders.

A hemorrhagic testis from 1 mouse with extensive myocarditis was ground in broth, passed through a Mandler regular filter and inoculated onto the chorioallantoic membrane, but no lesion was observed.

Transmission Experiments

The inoculations of heart tissue from 7 mice with myocarditis into a total of 74 healthy mice by the intravenous, intracerebral, or intraocular routes were without results. The myocardial tissue from the 8th mouse was injected into 4 normal mice by the intrapulmonary route, and into 5 by the intravenous route. Of the mice injected intravenously, 1 died within 24 hours and the heart was negative; another died within 10 days with both gross and microscopic evidence of myocarditis. Cultures of the heart muscle yielded no growth. The tissue was passed to 5 more mice. All died, however, as a result of *Bacillus typhi murium* infection within the incubation period of myocarditis. Three killed after 24 days had no evidence of disease. Of those inoculated by intranasal instillation, 1 died on the 18th and 1 on the 24th day, and although there was no gross evidence of myocarditis, it was present on histologic examination. The remaining 2 were killed on the 24th day and there was no evidence of disease. The inoculated animals that showed myocarditis had neither pleural nor testicular hemorrhage, and on subsequent passages the mice of

this series died from infection with *B. typhi murium*. It is probable that this myocarditis was attributable to *B. typhi murium* that was present in a small number of mice purchased for transmission experiments. This infection had not been encountered in our colony and the inoculated animals were kept in a different room.

More recently, numerous mice inoculated with blood from pleural fluid taken at autopsy died within a few days with septicemia produced by a bipolar organism that was not identified; the surviving mice showed no change. The organism did not reproduce any manifestations of the disease.

Discussion

The following possibilities were considered in respect to etiology of this disease: (a) infection, (b) degeneration of cardiovascular system, (c) vitamin deficiency, (d) hormonal factors.

The epidemiologic considerations do not suggest an infectious disease and the remarkable sex incidence would also argue against it. Nevertheless, numerous cultural and transmission experiments were made, but without success. Pleuropneumonia-like organisms were not found. Most inoculated animals remained healthy, but in one series 3 of 9 mice injected died with myocarditis, accompanied by *B. typhi murium*, which occurred in the mice purchased for inoculation but not in those of our animal colony. The negative transmission experiments are against causation of this disease by a virus, but it is possible that the proper requisites for successful transmission of the disease have not been met.

The myocardial changes observed were both inflammatory and degenerative in character, and although it is possible that the degenerative changes were primary, the reverse seems more likely. Alterations in the blood vessels of the heart were rare and scant. Involvement of the auricles was more conspicuous than that of the ventricles, and hemorrhage was apparently due to rupture of the auricles. There seems to be no similar disturbance known in man, though in absence of the testicular and pleural hemorrhage the cases would have been designated as isolated (Fiedler's) myocarditis (*cf.* Saphir [3]).

In these mice there was no generalized hemorrhagic tendency and there are no reasons to suspect vitamin C or vitamin K deficiencies. Deficiency of vitamin B complex, however, should be considered. The cardiac changes in vitamin B_1 (thiamin chloride) deficiency are not well known. They were adequately described by Wenckebach,[4] who regarded hydropic degeneration as the outstanding microscopic change,

and dilatation of the right heart, particularly that of the pulmonary conus, as the outstanding gross change. It has not been possible to reproduce the anatomic changes of the disease with certainty nor to correlate gross and microscopic changes with the clinical manifestations of the beriberi heart. Recently, Thomas, Mylon and Winternitz [5] produced myocardial degeneration with subsequent fibrosis by keeping rats on vitamin B_1 deficiency and on a low potassium diet. Observations made by Dock [6] and by Smith and Furth [7] suggest that myocardial fibrosis may be a late manifestation of human vitamin B_1 deficiency, but the cardiac changes in mice here described are those of acute or subacute myocarditis and not of myocardial and endocardial fibrosis.

Goettsch and Pappenheimer [8] reported the production of degeneration of the voluntary muscle of rabbits and guinea pigs when given a diet lacking in vitamin E. More recently, Pappenheimer [9] has found that the offspring of female mice maintained on a vitamin E-deficient diet showed extensive degeneration of the voluntary muscle fibers, characterized chiefly by hyaline necrosis and infiltration with polymorphonuclear leukocytes. No mention was made of the changes in the heart.

Myocarditis was stated by Lenke and Loewe [10] to be common among mice, but since the occurrence of pleural and testicular hemorrhages was not mentioned, it is not likely that the disease described by them is the same as that studied by us.

Since the disease affected only males during the period of sexual activity, some hormonal factors may be operating. Microscopic examination of the testes, adrenals and hypophysis from several cases, however, showed no conspicuous deviation from normal.

Summary

A disease complex not hitherto reported is described in mice. It is characterized by sudden death with myocarditis and exsanguinating pleuropericardial hemorrhage, accompanied almost invariably by testicular hemorrhage. It is found in about 1.2 per cent of the mice of our colony dying spontaneously and affects healthy, well developed and well nourished male mice, perhaps with rare exceptions. The disease is most common between 10 and 19 months of age, with a peak at 14 months. Epidemiologic observations, transmission experiments and cultural studies failed to support the opinion that the disease is infectious.

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REFERENCES

1. Anitschkow, N. Experimentelle Untersuchungen über die Neubildung des Granulationsgewebes im Herzmuskel. *Beitr. z. path. Anat. u. z. allg. Path.*, 1913, **55**, 373–415.
2. Ehrlich, J. C., and Lopan, Bernard. The Anitschkow "myocyte." *Arch. Path.*, 1939, **28**, 361–370.
3. Saphir, Otto. Myocarditis; a general review, with an analysis of 240 cases. *Arch. Path.*, 1941, **32**, 1000–1051; 1942, **33**, 88–137.
4. Wenckebach, K. F. Das Beriberi-herz; Morphologie, Klinik, Pathogenese. Berlin, 1934.
5. Thomas, R. M.; Mylon, E., and Winternitz, M. C. Myocardial lesions resulting from dietary deficiency. *Yale J. Biol. & Med.*, 1940, **12**, 345–360.
6. Dock, W. Marked cardiac hypertrophy and mural thrombosis in the ventricles in beriberi heart. *Tr. A. Am. Physicians*, 1940, **55**, 61–70.
7. Smith, J. J., and Furth, J. Chronic changes in beriberi heart and their relation to isolated (Fiedler's) myocarditis. *Arch. Int. Med.* (In press.)
8. Goettsch, Marianne, and Pappenheimer, A. M. Nutritional muscular dystrophy in the guinea pig and rabbit. *J. Exper. Med.*, 1931, **54**, 145–165.
9. Pappenheimer, A. M. Muscular dystrophy in mice on vitamin E-deficient diet. *Am. J. Path.*, 1942, **18**, 169–181.
10. Lenke, S. E., and Loewe, Leo. Cardiac lesions resembling Aschoff bodies in mice. *Am. J. Path.*, 1941, **17**, 857–859.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 23

All sections were stained with hematoxylin and eosin. The magnifications are approximate.

FIG. 1. Heart of mouse Rfe 189 showing advanced myocardial and endocardial infiltration, mainly by polymorphonuclear leukocytes with hemorrhage. × 100.

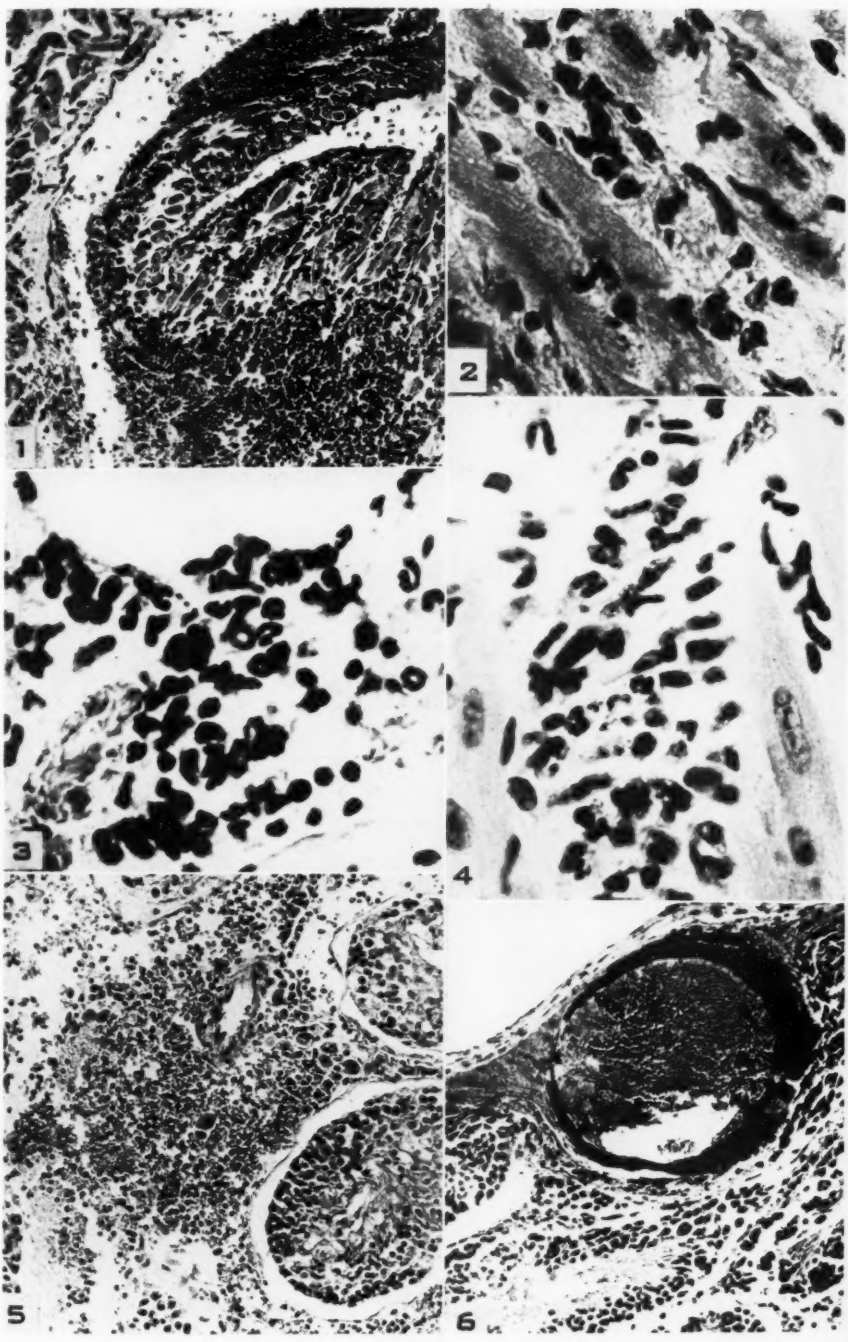
FIG. 2. Higher magnification of the myocardial changes in mouse Rfe 189 showing edema and infiltration by lymphoid cells. × 800.

FIG. 3. Infiltration of the epicardial fat in mouse R.Rff 396, continuous with that of the epicardial and myocardial infiltration. Most cells in this field are polymorphonuclear leukocytes; several are lymphoid cells and monocytes. × 800.

FIG. 4. Myocardial infiltration in the same mouse with predominantly large mononuclear leukocytes and young fibroblastlike cells. × 800.

FIG. 5. Advanced interstitial hemorrhage in the testis of mouse Rfg 342 with large numbers of polymorphonuclear leukocytes among the erythrocytes. × 100.

FIG. 6. Advanced calcification of a medium-sized artery beneath the capsule of the testis of mouse R.Rff 396. On the left side the wall of the artery is very thin and hemorrhage extends beneath the capsule. × 100.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